

- The effects Ohta et al. (2001) described in mice after single intraperitoneal injections on long-term potentiation in hippocampal slices may be significant to any derivation of acute-exposure risk assessment, particularly considering the critical nature of the effect and its implications for complex cognitive function. However, the intraperitoneal route of administration makes extrapolation of these findings to humans difficult.
- Although the exposure concentrations (0.2-200 ppm) used in the subchronic study of rats by Poon et al. (2002) are low and thus would be of interest, small sample sizes used in components of this study may have precluded the ability to detect effects; histologic changes are hard to interpret given measurement at a single time point. Thus, the absence of effects reported in this study may reflect experimental inadequacies rather than representing actual no-observed-adverse-effect levels.
- The two studies by Kilburn (2002a,b) of chronic environmental exposures in humans have major limitations, including potential for bias, inconsistency of effects, absence of appropriate comparisons, and others as noted above. For these reasons, it is not clear that the studies can be used in the trichloroethylene risk assessment.
- A study by Reif et al. (2003) reporting that low (and high) concentrations of trichloroethylene affect memory, as well as a potentiation of such effects by alcohol (an interaction supported by the experimental literature) has limitations that include potential misclassification of exposures and bias, and thus must be interpreted with caution.
- Certainly of potential relevance to risk assessment are studies that suggest protracted effects of trichloroethylene after cessation of exposure, such as described by Oshiro et al. (2004) for dopaminergic systems and by Haglid et al. (1981) for alterations in protein levels in multiple brain regions.

One thing made clear by any assessment of the trichloroethylene literature as it relates to the nervous system is the paucity of data available to define the extent of its neurotoxicity and the parameters and conditions of exposure under which it occurs. For example, studies of chronic exposure are limited. The realities of evaluating the impact of human environmental exposures generally mean that measures of trichloroethylene exposures in such studies are estimated and not empirically determined, leaving open the possibility of misclassification, a problem that can increase or decrease the probability of detecting effects. In some other human studies, the effects are confounded by involvement of the subjects in litigation. When experimental studies were carried out, they were generally not lifetime studies and the extent to which they evaluated behavioral and neurological function was limited, because most of the emphasis was on carcinogenicity.

Recommendation: Long-term studies, human and experimental, are critically needed to evaluate the effect of trichloroethylene on the central nervous system. For human studies, measurement or better estimates of exposure are necessary.

Another gap in the literature is the extent to which development represents a period of enhanced susceptibility to the neurotoxic effects of trichloroethylene. The only comparative data available at the current time appears to be from the study of Moser et al. (1999) examining one metabolite of trichloroethylene, dichloroacetic acid, where the evidence in support of enhanced susceptibility of younger rats is limited. Statements of greater sensitivity of children than of

adults exposed to trichloroethylene are also described by White et al. (1997), but again the supporting data are not presented.

The one study published to date clearly demonstrates an enhanced sensitivity of aging rats relative to young rats to the effects of trichloroethylene, measured in that study as changes in heart rate (Arito et al. 1994). These changes were shown to be due to changes in pharmacokinetics with age, with older rats exhibiting higher brain concentrations of trichloroethylene as well as longer exposure (delayed clearance) to those doses. One would predict that this enhanced toxicity should generalize to other behavioral and neurological consequences of trichloroethylene as well, because functionally it represents a higher dose to the brain, particularly if peak blood trichloroethylene concentrations are critical to adverse effects (Boyes et al. 2003). This also means that aging and exposure duration may be related in chronic exposure scenarios in humans.

Recommendation: More research is needed to assess different life stages at which humans might be more susceptible to the neurotoxic effects of trichloroethylene.

Another area of interest is the possibility of permanency versus reversibility of effects and the conditions under which this could occur. To date, the evidence is conflicting and undoubtedly would reflect the parameters of exposure, but some studies document protracted effects of trichloroethylene on the nervous system (e.g., Haglid et al. 1981; Oshiro et al. 2004; Crofton et al. 1993). It is clear from studies reported many years ago that acute exposures to high concentrations of trichloroethylene, in occupational or experimental contexts, can produce permanent changes in the nervous system. What is not yet known is the exposure conditions, particularly repeated exposures, under which effects would no longer be reversible. A related issue is whether effects of exposure can be progressive even after that exposure has terminated.

Recommendation: Additional research examining the extent to which observed effects are permanent versus reversible would be of relevance to risk evaluation.

Despite the associations of occupational exposures with memory loss and other cognitive deficits, the nature of such effects and the exposure conditions with which they can be associated have not been elaborated. A report by White et al. (1997), despite its deficiencies, clearly shows common effects on complex cognitive functions across three different populations exposed to trichloroethylene environmentally. Experimental studies have been less clear about such effects, but the extent to which this has been addressed is limited and, in some published reports, is not interpretable with respect to outcome.

A related function that clearly seems to be affected by trichloroethylene is motor function, as has been demonstrated in experimental studies as well as in occupational cohorts. As with other behavioral functions, the trichloroethylene exposure conditions under which such effects occur are not yet known. It may be important to define such conditions, particularly if, as suggested by other reports, trichloroethylene might contribute to neurodegenerative disorders such as Parkinson's disease. The earliest signs of motor dysfunction could serve as biomarkers of such a contribution.

Many neurological and behavioral disorders represent complex multifactorial etiologies. Given the broad spectrum of its effects across behavioral domains as well as neurotransmitter systems (and other as yet unknown mechanisms), it is possible that trichloroethylene may

contribute as a risk factor to other neurodegenerative and behavioral diseases or dysfunctions, acting in conjunction with other risk modifiers that may include genetic background (P-450 polymorphisms) and lifestyle factors (e.g., alcohol consumption), aging, and other factors that are undetermined.

Recommendation: Studies of additional functional end points, including cognitive deficits and motor and sensory function in response to chronic exposures to trichloroethylene would be of value to risk assessment.

Current evidence suggests the possibility of multiple mechanisms by which trichloroethylene may act, with the recognition that these mechanisms may also depend on the parameters of exposure. Extant literature already documents changes in long-term potentiation as well as alterations in functions of several neurotransmitter systems, a basis from which complex cognitive functions as well as other behavioral domains could be impaired.

Recommendation: Additional research is required to elucidate the underlying modes of action of trichloroethylene-induced neurotoxicity.

7

Respiratory Tract Toxicity and Cancer

This chapter reviews information on the effects of trichloroethylene on the respiratory system, particularly information generated since the U.S. Environmental Protection Agency released its draft health risk assessment (EPA 2001b). In keeping with the committee's charge, the chapter focuses on hazard characterization and the mode of action for trichloroethylene toxicity, assessing the available information from in vitro, animal, and human studies. Cancers of the respiratory tract are also considered.

RESPIRATORY TRACT TOXICITY

In Vitro Studies

Trichloroethylene, tested in swine trachea in vitro for its effects on smooth muscle contraction and epithelial release of prostanoids, did not alter the basal tone of tracheal smooth muscle but did potentiate the muscle contractile responses to acetylcholine and histamine in a concentration-dependent manner. Trichloroethylene increased epithelial prostaglandin E₂ release and decreased acetylcholinesterase activity. Such responses are consistent with the reported effects of trichloroethylene exposure on increasing airway hyperresponsiveness and asthma (Chen et al. 2005).

In Vivo Studies

The time course of trichloroethylene-induced pulmonary injury was followed in CD-1 male mice exposed to [¹⁴C]trichloroethylene in a single oral dose of 2,000 mg/kg (Forkert and Birch 1989). Clara cells of the bronchiolar epithelium showed necrotic changes within 1 hour of dosing and most Clara cells were severely vacuolated by 24 hours. Twenty percent of the lung burden at 4 hours was covalently bound. The study indicates that the highly metabolic Clara cells are targets of trichloroethylene toxicity in the respiratory tract.

Human Studies

There are few reports of non-cancer pulmonary toxicity in trichloroethylene-exposed humans. A study of respiratory findings in gun factory workers exposed to multiple solvents indicated significant effects of smoking and exposure to solvents, with smoking having the most important effect on asthma-related symptoms. Trichloroethylene was only one of many solvents to which the workers were exposed (Cakmak et al. 2004).

Toxicokinetics and Mode of Action

Pulmonary toxicity induced by trichloroethylene is associated with cytochrome P-450-dependent bioactivation to reactive metabolites (see Figure 7-1). The predominant pathway of

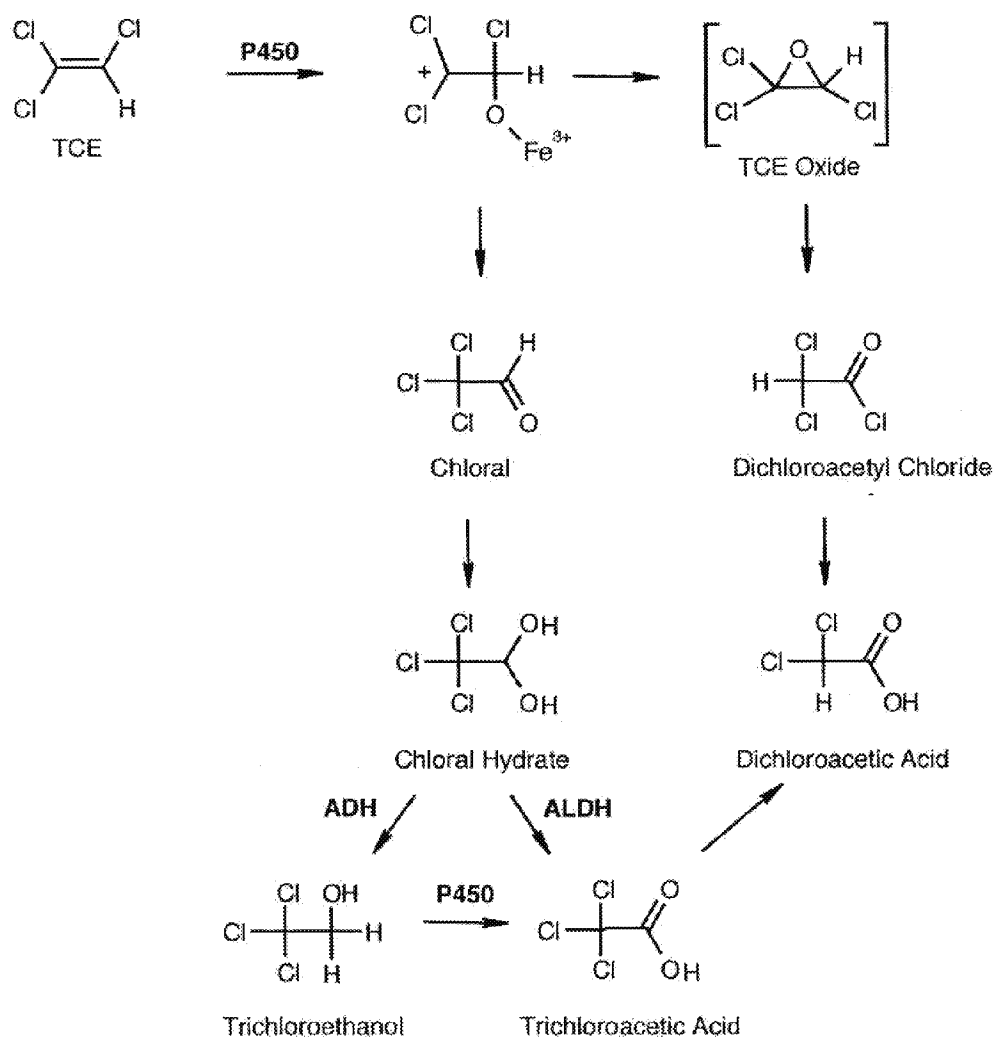


FIGURE 7-1 Proposed scheme of trichloroethylene metabolism. Abbreviations: TCE, trichloroethylene; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase. Source: Forkert et al. 2005. Reprinted with permission; copyright 2005, American Society for Pharmacology and Experimental Therapeutics.

trichloroethylene metabolism is oxidation via the cytochrome P-450 system, mainly by CYP2E1, although other P-450 enzymes including CYP1A1/2, CYP2B1/2, CYP2C11/6, and CYP2F have been implicated (Guengerich et al. 1991; Nakajima et al. 1992a; Forkert et al. 2005). Oxidative metabolism of trichloroethylene yields the primary metabolites chloral, trichloroethylene oxide, and dichloroacetyl chloride. Chloral, a predominant metabolite of trichloroethylene, is rapidly converted to chloral hydrate, which then undergoes oxidation and reduction by aldehyde-dehydrogenase and alcohol-dehydrogenase enzymes to form trichloroacetic acid and trichloroethanol (Green and Prout 1985; Dekant et al. 1986b). Clara cells isolated from the mouse lung are known to efficiently metabolize trichloroethylene to chloral and trichloroacetic acid. Recent studies of recombinant (r) cytochrome P-450s and rodent and human lung microsomes revealed that rat and human rCYP2E1, rCYP2F, and rCYP2B1 were all capable of mediating trichloroethylene metabolism to chloral hydrate (Forkert et al. 2005). Rat rCYP2E1 exhibited greater affinity than rat rCYP2F4 and rCYP2B1 and human rCYP2E1. More recently, the same investigators (Forkert et al. 2006) suggested that CYP2F2 might play a greater role than CYP2E1 in the metabolism of trichloroethylene in the mouse lung. Treatment of CYP2E1-null and wild-type mice with trichloroethylene led to bronchiolar damage that correlated with the formation of dichloroacetyl adducts in the Clara cells. These findings provide evidence for bioactivation of trichloroethylene within the Clara cells, predominantly involving CYP2F2, that correlates with bronchiolar cytotoxicity.

The rates of chloral hydrate production in human lung microsomes were low and were detected in only three of eight subjects (Forkert et al. 2005). Furthermore, the rates of chloral hydrate production were substantially higher in murine than in human lung. Alcohol dehydrogenase, the enzyme responsible for metabolizing chloral to trichloroethanol, is known to be at low concentrations in lung tissue (Sorokin 1970), with chloral being the major metabolite in these cells. Trichloroethanol glucuronide, a major metabolite in the liver, is not formed in the Clara cells due to lack of glucuronyltransferase (Odum et al. 1992). Recent studies of mice treated intraperitoneally with high doses (500-1,000 mg/kg) of trichloroethylene found dichloroacetyl protein adducts in Clara cells (Forkert et al. 2006). The proximate toxicant for the Clara cell, whether chloral, dichloroacetyl chloride, or another metabolite, is still under study.

Exposure to trichloroethylene occurs mainly through inhalation and oral routes and rapid absorption occurs by both routes. Absorption of inhaled trichloroethylene is both rapid and extensive. Regardless of the route of exposure, unmetabolized trichloroethylene is eliminated by exhalation. Consequently, pulmonary airways are exposed to trichloroethylene regardless of the route of exposure. However, the amount of pulmonary exposure to trichloroethylene after an oral exposure is dose dependent and will be high only after an oral dose exceeds the capacity of the liver to metabolize the trichloroethylene. After inhalation exposure, trichloroethylene is rapidly absorbed through the alveolar endothelium due to a high blood-gas partition coefficient. However, the blood-gas partition coefficient in humans is 1.5- to 2.5-fold lower than that in mice and rats, respectively, which suggests that delivery of trichloroethylene to the circulatory system for translocation to target organs may be significantly less efficient in humans. This factor should be taken into account when using animal data in risk assessment analysis for trichloroethylene (Sato et al. 1977; Prout et al. 1985; Clewell et al. 1995).

The Clara cells develop vacuoles (Forkert and Birch 1989; Odum et al. 1992) after exposure to trichloroethylene and proliferate with continued exposure (Green et al. 1997b). Considering the site of induced tumors in mice and the observed toxicity sites, it appears that the Clara cell is the most sensitive site in the respiratory tract with respect to the toxicity of inhaled

trichloroethylene. CYP2E1 and CYP2F2 are highly concentrated in mouse Clara cells (Buckpitt et al. 1995; Forkert et al. 1995) and the presence of dichloroacetyl protein adducts in these same cells (Forkert et al. 2006) suggests that the bioactivation of trichloroethylene takes place in these cells. The low CYP2E1 concentration in human Clara cells suggests that humans are not as sensitive as mice for the development of lung tumors as a result of trichloroethylene exposures at ambient levels. This hypothesis agrees with the results of most epidemiology studies (discussed later in this chapter), which do not indicate a strong association between trichloroethylene exposure and increased incidence of lung tumors.

Whereas trichloroethylene is both acutely toxic and carcinogenic to the mouse lung after exposure by inhalation, it is not carcinogenic in the rat lung and is markedly less toxic after acute exposure (Stewart et al. 1979; Fukuda et al. 1983; Maltoni et al. 1986, 1988; Davidson and Beliles 1991). The lack of toxicity or carcinogenicity in the lungs of mice after oral dosing is presumably due to extensive hepatic metabolism reducing the amount of trichloroethylene that reaches the lungs. Adverse effects on human lungs have not been reported. The primary effects of trichloroethylene on mouse lungs in all studies have been morphologic and biochemical changes in the nonciliated Clara cells (Forkert 2001). The only other toxicologic responses noted in the lung after exposure to trichloroethylene were fibrosis in the mouse (Forkert and Forkert 1994) and a decrease in surfactant phospholipid after exposure of rats and mice to high doses (3,000 mg/kg) of trichloroethylene (Scott et al. 1988). Loss of cytochrome P-450 activity and morphologic recovery of the Clara cells with repeated daily exposure of mice to trichloroethylene suggest that loss of metabolic capacity in these cells is an adaptive mechanism (Lewis et al. 1984; Forkert et al. 1985, 2005; Odum et al. 1992).

RESPIRATORY TRACT CANCER

Animal Studies

Animal carcinogenicity studies are summarized in Table 7-1. Trichloroethylene inhalation exposure caused an increased incidence of pulmonary tumors in mice (Fukuda et al. 1983; Maltoni et al. 1986, 1988) but not in rats and hamsters (Henschler et al. 1980; Fukuda et al. 1983; Maltoni et al. 1986, 1988). Oral administration of trichloroethylene did not result in lung tumors (NCI 1976; Van Duuren et al. 1979; Henschler et al. 1984; Maltoni et al. 1986; NTP 1988, 1990) in any species studied. Details of those studies follow.

Inhalation Exposure

The inhalation studies by Fukuda et al. (1983) included exposing female ICR mice and female Sprague-Dawley rats to trichloroethylene at 0, 50, 150, or 450 ppm for 7 hours/day, 5 days/week for 104 weeks followed by an observation period of 3 weeks. They observed a threefold increase in lung tumors per mouse in those exposed to the two higher concentrations but saw no increase in lung tumors in the rats.

In the studies by Maltoni et al. (1986, 1988), male and female Swiss mice and Sprague-Dawley rats were exposed to trichloroethylene at 0, 100, and 600 ppm for 7 hours/day, 5 days/week for 8 weeks and for 104 weeks (rats only). B6C3F₁ and Swiss mice were also

TABLE 7-1 Animal Carcinogenicity Studies of Trichloroethylene

Reference	Animals (Sex)	Exposure Route	Stabilizers	Doses/Exp Conc.	Exposed	Results
Fukuda et al. 1983	ICR mice (F)	Inhalation, 7 h/day, 5 days/week, 104 wk, hold 3 wk	Epichlorohydrin	0, 50, 150, 450 ppm	50/group	Threefold increase in lung tumors in mice at two higher concentrations; no increase in any tumors in rats.
	S-D rats (F)					
Maltoni et al. 1986, 1988	S-D rats (M, F)	Inhalation, 7 h/day, 5 days/week, 104 wk; hold until death	No stabilizer	0, 100, 300, 600 ppm	130-145/group	Increase in tumors of testis and kidney (high dose only) in males.
	Swiss mice (M, F) B6C3F ₁ mice	Inhalation, 7 h/day, 5 days/week, 78 wk; hold until death	No stabilizer	0, 100, 300, 600 ppm	90/group	Excess lung tumors in both strains; liver tumors in male Swiss mice.
Henschler et al. 1980	Wistar rats (M, F)	Inhalation, 6 h/day, 5 days/week, 18 months	Triethanolamine	0, 100, 500 ppm	30/group	No increase in any tumors, except increase in lymphomas in female mice.
	Syrian hamsters (M, F) NMRI mice	Inhalation, 6 h/day, 5 days/week, 18 months				
Van Duuren et al. 1979	Swiss mice (M, F)	Gavage, 1/wk, 89 wk	Unknown	0, 0.5 mg	30/group	No excess tumors in lung, liver, or stomach.
	Osborne-Mendel rats (M, F)	Gavage, 5/wk, 78 wk, hold 110 wk (rats) or 90 wk (mice)	Epoxbutane, epichlorohydrin	0, 500, 1,000 mg/kg (rats) 0, 1,000, 3,000 mg/kg (mice)	50/group except 20/control	No excess pulmonary tumors; increase in liver tumors.
NTP 1988	4 strains of rats	Gavage, 1/day, 5 days/week, 103 wk	No stabilizer	0, 500, 1,000 mg/kg	50/group	No excess pulmonary tumors; some renal and testicular tumors in two strains.
NTP 1990	F344 rats (M, F)	Gavage, 1/day, 5/wk, 103 wk	No stabilizer	0, 500, 1,000 mg/kg (rats) 0, 1,000 mg/kg (mice)	50/group	No pulmonary tumors; increase in liver tumors.
	B6C3F ₁ mice (M, F)					
Maltoni et al. 1986	S-D rats (M, F)	Gavage, 1/day, 4-5 days/week, 56 wk; hold until death	No stabilizer	0, 50, or 250 mg/kg	30/group	No excess tumors.

Abbreviations: F, female; M, male; S-D, Sprague-Dawley.

exposed for 78 weeks to trichloroethylene at 0, 100, 300, or 600 ppm. Animals were held for observation until spontaneous death. Excess tumors were not observed in either species after the 8-week exposures. Excess pulmonary tumors were observed in both strains of mice after 78 weeks of exposure, but no excess pulmonary tumors were observed in rats after the 104-week exposures.

Henschler et al. (1980) exposed NMRI mice, WIST rats, and Syrian hamsters of both sexes to trichloroethylene at 0, 100, or 500 ppm for 6 hours/day, 5 days/week for 18 months. They observed no pulmonary tumors in any of the species. The trichloroethylene used was technical grade and contained traces of epichlorohydrin and 1,2-epoxybutane—two known carcinogenic compounds used as stabilizers.

Gavage Exposure

Van Duuren et al. (1979) tested trichloroethylene among 14 other halogenated compounds for carcinogenicity in Swiss mice. The mice (both sexes) were administered trichloroethylene at 0.5 mg per intragastric intubation once a week for 622 days (89 weeks). No excess tumors were observed.

The National Cancer Institute (NCI 1976) and the National Toxicology Program (NTP 1988, 1990) reported three carcinogenicity studies of trichloroethylene administered by gavage to rats and mice. The first study (NCI 1976) used both sexes of Osborne-Mendel rats and B6C3F₁ mice. Animals were dosed with trichloroethylene at approximately 500 or 1,000 mg/kg (rats) or approximately 1,000 or 2,000 mg/kg (mice), 5 times/week for 78 weeks. Rats were observed for 110 weeks and mice were observed for 90 weeks. No increase in pulmonary tumors was observed in either species, but the study was not considered valid because of early mortality among the rats. The trichloroethylene was technical grade and was stabilized with epichlorohydrin and 1,2-epoxybutane. In a later study (NTP 1988), trichloroethylene stabilized by diisopropylamine was administered orally to four strains of rats at 0, 500, or 1,000 mg/kg per day, 5 days/week, for 103 weeks. No pulmonary tumors were observed, but the study was considered inadequate because of chemical toxicity and early mortality in the rats. The third study (NTP 1990) was conducted in male and female F344 rats and B6C3F₁ mice with epichlorohydrin-free trichloroethylene. Doses of 500 or 1,000 mg/kg in rats and 1,000 mg/kg in mice were given by gavage 5 days/week for 103 weeks. No pulmonary tumors were observed. The study was considered adequate for demonstrating no carcinogenicity in female rats, but the male rats did not survive long enough to test adequately for carcinogenicity.

Epidemiology Studies

Because it was not possible to provide a comprehensive evaluation of the epidemiologic evidence on trichloroethylene and different cancers, the committee borrowed a previously compiled summary of the epidemiologic evidence on lung cancer from the Institute of Medicine (IOM 2003) to give some perspective on the evidence for lung cancer (see Table 7-2). The list was updated with one study published since the IOM report. Epidemiology studies do not indicate an increase in pulmonary tumors in association with trichloroethylene exposure, except for the new study in Denmark (Raaschou-Nielsen et al. 2003), which was done on a cohort of

TABLE 7-2 Epidemiologic Data on Lung Cancer and Exposure to Trichloroethylene

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% confidence interval)
Cohort Studies—Incidence			
Raaschou-Nielsen et al. 2003	Workers in Denmark		
	Males		
	<1 yr	181	1.6 (1.4-1.9)
	1-4.9 yr	193	1.3 (1.1-1.5)
	≥5 yr	185	1.4 (1.2-1.6)
	Females		
	<1 yr	28	2.5 (1.6-3.6)
	1-4.9 yr	25	1.6 (1.1-2.4)
	≥5 yr	20	1.6 (1.0-2.5)
Hansen et al. 2001	Biologically monitored workers in Denmark		
	Males, ever exposed	16	0.8 (0.5-1.3)
	Females, ever exposed	1	0.7 (0.01-3.8)
Blair et al. 1998	Aircraft-maintenance workers in Utah		
	Males		
	No exposure	22	1.0 (0.5-1.9)
	<5 unit-yr	24	1.0 (0.6-2.0)
	5-25 unit-yr	11	0.8 (0.4-1.6)
	>25 unit-yr	15	0.8 (0.4-1.7)
	Females		
	No exposure	0	—
	<5 unit-yr	1	0.6 (0.1-5.3)
	5-25 unit-yr	0	—
	>25 unit-yr	0	—
Anttila et al. 1995	Biologically monitored workers in Finland		
	Entire period since first measurement	25	0.92 (0.59-1.35)
	0-9 yr	11	1.19 (0.59-2.13)
	10-19 yr	9	0.67 (0.30-1.26)
	≥20 yr	5	1.11 (0.36-2.58)
	Mean personal U-TCA concentration		
	<100 μmol/L	16	1.02 (0.58-1.66)
	100+ μmol/L	7	0.83 (0.33-1.71)
Cohort Studies—Mortality			
Boice et al. 1999	Aircraft-manufacturing workers in California		
	All exposed factory workers	78	0.76 (0.60-0.95)
	Duration of potential exposure (routine or intermittent)		
	<1 yr	66	0.85 (0.65-1.13)
	1-4 yr	63	0.98 (0.74-1.30)
	≥5 yr	44	0.64 (0.46-0.89)
Blair et al. 1998	Aircraft-maintenance workers in Utah		
	Males		
	No exposure	51	1.0 (0.7-1.6)
	<5 unit-yr	43	1.0 (0.6-1.6)
	5-25 unit-yr	23	0.9 (0.5-1.6)
	>25 unit-yr	38	1.1 (0.7-1.8)

TABLE 7-2 *Continued*

Reference	Study Population	Exposed Cases	Estimated Relative Risk (95% confidence interval)
Morgan et al. 1998	Females		
	No exposure	2	0.4 (0.1-1.6)
	<5 unit-yr	2	0.6 (0.1-2.4)
	5-25 unit-yr	11	0.6 (0.1-4.7)
	>25 unit-yr	2	0.4 (0.1-1.8)
	Aerospace workers in Arizona		
	Entire TCE-exposed cohort	97	1.10 (0.89-1.34)
	Cumulative		
	Low	45	1.49 (1.09-1.99)
	High	52	0.90 (0.67-1.20)
Greenland et al. 1994	Peak: medium and high versus low and no exposure	64	1.07 (0.82-1.40)
	White male transformer-assembly workers, ever exposed	NA	1.01 (0.69-1.47)
Wilcosky et al. 1984	White male rubber-industry workers in Ohio, cumulative exposure of more than 1 yr	11	0.64

Abbreviations: NA, not available; TCE, trichloroethylene; U-TCA, urinary trichloroacetic acid.
Source: Adapted from IOM 2003.

more than 40,000 blue-collar workers in 347 Danish companies where trichloroethylene was used. The standardized incidence ratio did not increase with duration of exposure, and was highest among workers with less than 1 year of exposure, particularly among females (SIR = 2.5). The authors suggested that smoking might be a confounding factor for cancers known to be related to tobacco use because of the lower socioeconomic status of the cohort and the higher prevalence of smoking among the least educated groups in Denmark. The authors also estimated that only 41% of the cohort were likely to have been exposed to trichloroethylene on the job, because the only marker of exposure used was employment in a blue-collar job at a trichloroethylene-using company. The large excess of lung cancer among females with the shortest exposures argues strongly against a causal interpretation for these findings.

Mode of Action

When trichloroethylene is inhaled, a large portion is absorbed (a greater percentage of what is inhaled is absorbed at lower concentrations) and is rapidly distributed throughout the body in the bloodstream. The liver is the major site of metabolism, but the fact that inhaled trichloroethylene causes lung tumors in mice, whereas orally administered trichloroethylene does not, suggests that pulmonary metabolism may play a role in lung tumor formation in mice. Trichloroethylene is not mutagenic but some of its metabolites are. Because trichloroethylene is lipid soluble, one would expect the compound to be delayed long enough in crossing the lung tissue into the blood to be partially metabolized by metabolically active epithelial cells (Clara cells and Type II cells) (Gerde et al. 1993). The metabolic activity of such cells toward several lipophilic xenobiotics is known to be higher in mouse cells than in rat or hamster cells, which

may be part of the reason for the greater susceptibility of mouse tissue than of tissue in rats and hamsters.

Some investigators have reported that the capacity of the mouse lung to metabolize trichloroethylene to the mutagenic metabolite chloral is an order of magnitude higher than in the rat lung, whereas human lung samples have no detectable activity (Green et al. 1997b). The CYP450 (2E1) enzyme responsible for this oxidation is present in high amounts in the Clara cells of mice and in lesser amounts in Clara cells of rats. The enzyme was not detected in human Clara cells, although mRNA for the enzyme has been detected and variable amounts of CYP2E1-like metabolism has been observed in human lung microsomes (Forkert et al. 2001).

The Clara cell is the site of toxicity induced by inhalation of trichloroethylene in mice. A number of acute toxicity studies have shown that trichloroethylene selectively targets the mouse lung Clara cell, suggesting the role of Clara cells in the development of mouse lung tumors (Buckpitt et al. 1995; Forkert and Forkert 1994). Although there is no direct evidence that mouse lung tumors are derived from Clara cells, the lack of other types of epithelial cells such as Type II cells responding by cell division suggests that Clara cells play a key role in the development of lung tumors in mice exposed to trichloroethylene. Similarly, differences in the metabolic capacity of Clara cells in mice and rats are consistent with species differences in toxicity and carcinogenicity (Green 2000). Clara cells are thought to be the cells of origin of some chemically induced mouse lung tumors (Sorokin 1970; Kauffman et al. 1979; Sato and Kauffman 1980; Kauffman. 1981; Thaete and Malkinson 1991; Palmer. 1985; Rasmussen et al. 1986; Rehm et al. 1991). However, evidence from antigenic staining for detailed morphologic characterization of the tumors is lacking.

ISSUES

Possible Modes of Action for Cancer and Non-Cancer Effects

Cytotoxicity and increased cell division form the basis of a plausible mode of action for trichloroethylene-induced lung tumors in mice. Both are known risk factors for mouse lung carcinogenesis because of significant background tumor incidences (Green 2000). In addition, chloral appears to have genotoxic potential (Salmon et al. 1995), although in the whole lung two studies failed to find evidence of DNA binding in mice exposed to trichloroethylene (Forkert and Birch 1989; Leuschner and Leuschner 1991). In conclusion, a number of known risk factors for the development of tumors, such as cytotoxicity, increased cell proliferation, and possibly aneuploidy, correlate well with the observed species-specific pulmonary carcinogenicity of trichloroethylene.

Relevance of Animal Studies to Humans

The acute responses believed to be causally related to the development of lung tumors in mice exposed to trichloroethylene have been attributed to the high metabolic capacity of the mouse lung Clara cells. Comparisons of the metabolic capacity of mouse, rat, and human lung tissue found that mouse lung microsomes metabolized trichloroethylene to chloral at a rate three-fold higher than the rat lung microsomes. A metabolic rate could not be detected in the human

lung (Green et al. 1997b). With an antibody to CYP2E1, the enzyme responsible for metabolism of trichloroethylene to chloral (Green et al. 1997b; Cruzan et al. 1997), the highest concentrations of the enzyme were found in the mouse lung. Significantly lower amounts were found in the rat lung. This enzyme could not be detected in the human lung in any cell types or in human lung sections by Western blotting (Green et al. 1997b). Other studies (Guengerich et al. 1991; Wheeler et al. 1992; Willey et al. 1996) reported the presence of CYP2E1 in human lung detectable only by reverse transcriptase-polymerase chain reaction. The total cytochrome P-450 content of the human lung is reported to be only 3.7% (27-fold lower than) that in the rat lung (Raunio et al. 1998). This is consistent with the lack of a measurable metabolic rate for trichloroethylene (Green et al. 1997b). Studies with recombinant cytochrome P-450s have revealed that, although rat and human rCYP2E1, rCYP2F, and rCYP2B1 were all capable of mediating trichloroethylene metabolism to chloral hydrate, the rat rCYP2E1 exhibited greater affinity than rat rCYP2F4 and rCYP2B1 and human rCYP2E1 (Forkert et al. 2005). These studies provide evidence supporting the role of CYP2E1, CYP2F, and CYP2B1 in the metabolism of trichloroethylene in the mouse lung. Studies with mouse and human lung microsomes indicated that the rates of chloral hydrate formation in human lung microsomes were low and were detected in only three of eight subjects (Forkert et al. 2005). Overall, the data suggest that the capacity of the human lung to metabolize trichloroethylene is 600-fold lower than the mouse lung (Green 2000).

The number of Clara cells and their morphology differ in human and rodent lungs, and this should also be considered in evaluating human risk from exposure to trichloroethylene. Clara cells differ significantly between rodents and among rodents and humans in number and structure (Reznik-Schuler 1976; Buckpitt et al. 1995). In mice, Clara cells are numerous and are spread throughout the airways, whereas in rats they are significantly fewer, particularly in the terminal bronchiolar region. In human lung, Clara cells are rare, as they are found in small numbers in the distal bronchioles. Mouse lung Clara cells are packed with endoplasmic reticulum, but human Clara cells are devoid of these membranes (Reznik-Schuler 1976; Buckpitt et al. 1995). The membranes of the endoplasmic reticulum in the Clara cell are the site of origin of the trichloroethylene-induced lesion in the mouse, consistent with the location of high concentrations of cytochrome P-450 enzymes that metabolize trichloroethylene in those membranes.

In summary, the metabolic data suggest that humans will be much less sensitive than mice to trichloroethylene-induced Clara cell toxicity and lung tumor development. One would not expect humans to develop lung tumors after exposure to ambient levels of trichloroethylene, which is in agreement with the results of epidemiology studies that show no association between trichloroethylene exposures and an increased incidence of lung tumors.

FINDINGS AND RECOMMENDATIONS

Trichloroethylene has been shown to induce lung tumors in rodents. It is well documented that the mode of action for this effect is localization of the metabolite chloral in Clara cells of the lungs and that pulmonary metabolism of trichloroethylene to chloral is species dependent. The weight of evidence indicates that rodents and humans differ significantly in their capacity to metabolize trichloroethylene in their lungs, with humans having less capacity to metabolize the compound. This is supported by the results of most epidemiologic studies of

occupational exposure to trichloroethylene, which do not show a strong association between trichloroethylene exposure and an increased incidence of lung tumors. Thus, pulmonary cancer is does not appear to be a critical end point in assessing human health risks of trichloroethylene.

8

Immunotoxicity

This chapter reviews information about the effects of trichloroethylene on the immune system, particularly information generated since the U.S. Environmental Protection Agency released its draft health risk assessment (EPA 2001b). Consideration is given to how the new information factors into previous assessments of the immunosuppressive and autoimmune effects of trichloroethylene, species differences, dose-response relationships, and mode of action information.

BACKGROUND

Immunotoxicity can be divided into two areas depending on whether the immune system is activated (such as in allergies or in chemical-induced autoimmune diseases) or suppressed by xenobiotics (foreign or nonendogenous chemicals, including drugs and environmental chemicals). Mammalian immune systems have innate and adaptive components that play important roles in resistance to infections and cancer. The immune systems of mammals are formed by primary lymphoid organs, including yolk sac, fetal liver, bone marrow, and thymus. Secondary lymphoid organs (e.g., lymph nodes, spleen, mucosa-associated lymphoid tissues) store differentiated cells that await activation by environmental antigens or undergo endogenous selection processes to discriminate self from non-self. T and B cells are activated in clonally restricted (antigen-specific) ways, and they demonstrate a memory response. One feature of innate immunity is that the responding cells (macrophages, natural killer cells, granulocytes) do not demonstrate clonal specificity. However, families of receptors have been identified (such as toll-like receptors) that allow innate cells to respond to certain families of environmental molecules or toxins (e.g., endotoxin). Xenobiotics may interfere with normal immune system homeostasis by affecting the formation of immune cells; modifying cell-to-cell interactions; modifying cell activation, proliferation, or differentiation; altering cell selection; and enhancing or suppressing the release of immune products such as cytokines, chemokines, antibodies, and complement factors.

The immunotoxicity of chemicals is evaluated in animal models, in in vitro studies, and occasionally in humans after occupational or environmental exposures. Environmental

epidemiology studies are often conducted to determine whether xenobiotic exposures are associated with disease. Because of the complexity of the innate and adaptive immune systems, no single assay can be used to study the potential toxicity of xenobiotics. Instead, a tiered approach has been developed and validated by several laboratories for studies in animals (Luster et al. 1988, 1992). Although there is no single immune assay or parameter that can be used to determine whether a xenobiotic exerts a toxic effect on the immune system, certain combinations of markers and functional assays can predict immunotoxicity (Luster et al. 1992). Additionally, the aforementioned assays are useful only for evaluating immunosuppressive chemicals. Few established assays exist for assessing hypersensitivity reactions of xenobiotics, and experimental models of autoimmunity are limited in their application and extrapolation to human autoimmune diseases.

ANIMAL STUDIES

Immunosuppression

The potential immunosuppressive and immunomodulating properties of trichloroethylene in acute, subchronic, and chronic exposures in animals have not been fully evaluated. Sanders et al. (1982) found that trichloroethylene at concentrations of 2.5-5 mg/mL (in drinking water for 4 or 6 months) resulted in suppression of humoral and cell-mediated immunity in female CD1 mice. Bone marrow stem cell activity was depressed at drinking water concentrations of 0.1-1 mg/mL. Male mice were less affected. Wright et al. (1991) found a depression in natural killer cell activity in the liver, decreased lipopolysaccharide lymphocyte mitogenesis, and decreased spleen weights after intraperitoneal exposures of Sprague-Dawley rats to trichloroethylene at 5 mmol/kg/day for 3 days. Natural killer cell activity in the liver was also depressed at 0.5 mmol/kg/day for 3 days. B6C3F₁ mice receiving the high-dose regimen also demonstrated spleen cell toxicity, and they were more sensitive than rats to the natural killer cell suppression in the liver, with effects observed at 0.05 mmol/kg/day for 3 days. Aranyi et al. (1986) found that acute exposures to various solvents decreased the host resistance responses to *Klebsiella pneumoniae*. In these studies, trichloroethylene was not evaluated, but a related solvent, perchlorethylene, had a small effect. Kauffmann et al. (1982) found that mice exposed to chloral hydrate at 1/10th (144 mg/kg over 14 days) and 1/100th of a median lethal dose (14.4 mg/kg over 14 days) had no changes in immune parameters. However a 90-day exposure to trichloroethylene at 0.07 and 0.7 mg/mL in drinking water produced a significant decrease in humoral immunity in female, but not male, mice. Park et al. (1993) found that trichloroethylene at 50-200 parts per million (ppm) increased infection in bacteria-challenged (*Streptococcus zooepidemicus*) mice.

Kaneko et al. (2000) determined that inhalation of high concentrations of trichloroethylene (500-2,000 ppm) for 8 weeks depressed the serum IgG in *mrl/lpr* mice and increased the formation of lymphoblastoid cells. Changes in T-cell subsets (helper to suppressor ratio) were detected at 2,000 ppm after 8 weeks of exposure. The significance of these findings is difficult to assess because the investigators used an autoimmune-prone mouse strain (*mrl/lpr*), which is not commonly used in studies of immunosuppression.

In summary, various studies indicate that exposures to moderate or high concentrations of trichloroethylene over long periods have the potential to produce immunosuppression in animal

models. There are important differences in the amounts and types of immunosuppression depending on species and gender.

Autoimmunity

Epidemiology and case studies revealed that solvents, including trichloroethylene, might be associated with certain human autoimmune diseases. These reports triggered investigators to evaluate the effect of trichloroethylene in animal models susceptible to the induction of autoimmune disease, most notably the MRL mouse. Autoimmune disease has not been reported in normal mice treated with trichloroethylene, although a small increase in autoantibodies has been noted in some studies (see below).

Findings in Rodents

Several laboratories have reported that trichloroethylene causes or exacerbates underlying autoimmune diseases in genetically susceptible MRL mice. Effects have been observed at doses as small as 0.1 mg/kg/day in drinking water for 4 weeks (Griffin et al. 2000a), which the authors calculated may be below the current threshold limit value of 50 ppm set by the American Conference of Industrial Hygienists. Recently, extensive mechanistic work has been performed and several biologically plausible hypotheses have been advanced (Khan et al. 1995, 2001; Gilbert et al. 1999, 2004; Griffin et al. 2000a,b,c; Blossom et al. 2004). Several studies focused on the need for metabolism of trichloroethylene to chloral or dichloroacetic acid to produce autoimmune-induced hepatitis in genetically susceptible mice (Griffin et al. 2000a,b) (see Chapter 4), a syndrome that bears potential mechanistic similarities to halothane-induced hepatotoxicity in rodents and humans. Evidence has been presented that trichloroethylene or its metabolites may activate T cells (Gilbert et al. 1999, 2004; Griffin et al. 2000a) and/or alter T cell regulation and survival (Blossom et al. 2004) associated with polyclonal disease, as detected by circulating anti-DNA and other antibodies in genetically susceptible mice. Theoretically, trichloroethylene metabolites may be increased with enhancers of the *CYP2E1* gene, and autoimmunity in MRL mice induced by trichloroethylene has been shown to be inhibited with *CYP2E1* inhibitors (Griffin et al. 2000b). Despite these hypotheses, the mechanism(s) by which trichloroethylene exacerbates autoimmune disease in MRL mice has not been elucidated and the relevance to human exposures and disease has not been established.

Gilkeson et al. (2004) administered trichloroethylene at 1,000-10,000 parts per billion (ppb) in drinking water to NZB/NZW mice for 26 weeks. They found an increase in anti-DNA antibodies with trichloroethylene at 1,000 ppb and an increase in kidney disease at 10,000 ppb. In the same study, B6C3F₁ mice developed a small increase in autoantibody production, but no kidney disease was detected.

White et al. (2000) found a lack of evidence for trichloroethylene-induced autoantibody production and systemic-lupus-erythematosus-like disease in Brown Norway rats when trichloroethylene was given by oral gavage 5 days a week for 6 weeks at 100-400 mg/kg.

Changes in Hematologic Parameters in Dogs

Hobara et al. (1984) found that acute inhalation exposure to trichloroethylene (200 and 500 ppm) or intravenous injection (50 mg/kg) in beagles produced a transient decrease in circulating leukocytes, most notably neutrophils that rebounded to near control concentrations after several hours.

HUMAN STUDIES

Following is a brief qualitative review of some human studies that have investigated trichloroethylene in relation to immunologic end points. It is provided to give a perspective on some of the important areas to be pursued as part of the risk assessment for trichloroethylene. A more thorough review of the epidemiologic studies in terms of methods, exposures, and results are necessary to fully characterize the immunologic hazards posed by trichloroethylene (see Chapter 2 for guidance on how this should be done).

Immunosuppression and Immunomodulation

Byers et al. (1988) reported on a human leukemia cluster putatively exposed to high concentrations of trichloroethylene and other solvents via contaminated drinking water. There were long-term alterations in peripheral blood T-cell subsets in the family members of those with leukemia, which suggests of an immunologic abnormality. There were increases in infections as well as an increased number of autoantibodies in this cohort. Lehman et al. (2002) found that children exposed in utero to volatile organic compounds, including trichloroethylene, had a shift toward TH₁ γ -interferon-producing T cells analyzed 6 hours after birth. These two studies suggest that immunologic changes may be seen in solvent- or trichloroethylene-exposed humans, although it has been difficult to quantify the exposures.

Autoimmunity

Numerous investigators have found an association between exposure to organic solvents, including trichloroethylene, and the human autoimmune diseases scleroderma (Saihan et al. 1978; Lockey et al. 1987; Waller, et al, 1994; Bovenzi et al. 1995, 2004; Nietert et al. 1998, 1999; Pandey and Takeuchi 1999; Pandey et al. 2001; Czirjak and Kumanovic 2002; Garabrandt et al. 2003; Pandey 2004) and Stevens-Johnson Syndrome (Pantucharoensri et al. 2004). The risk of scleroderma may be correlated with particular CYP2E1 and CYP2C19 polymorphisms (Povey et al. 2001), suggesting that trichloroethylene metabolism could be important in this disease. However, more research is needed to elucidate this possibility.

ISSUES FOR IMMUNOTOXICITY RISK ASSESSMENT

Extrapolation of Animal Data to Humans

A biologically plausible mechanism has been hypothesized for trichloroethylene-induced systemic autoimmunity and autoimmune hepatitis that involves the bioactivation of trichloroethylene to chloral in genetically susceptible mice. This mechanism might explain clonally restricted diseases, such as autoimmune-induced hepatitis, but does not explain polyclonal diseases. Chloral has been shown to bind to circulating proteins leading to an alteration in self that resulted in autoantibody formation and a chemically induced autoimmune syndrome. It is unclear whether this mechanism exists in humans, although it is notable that people with polymorphisms in CYP2E1 appear to have a higher risk of solvent-induced autoimmune disease. More studies are needed to incorporate aspects of innate and adaptive immune responses to study this and other proposed mechanisms of trichloroethylene-induced autoimmune diseases in humans.

Animal studies indicate that chronic exposure to trichloroethylene at moderate to high concentrations might have the potential to produce immunosuppression in animal models. However, there is no evidence to suggest that trichloroethylene is immunosuppressive in humans.

A potential biomarker for trichloroethylene exposure has been identified in mouse studies—namely, a chloral-protein adduct has been detected in tissues of trichloroethylene-treated mice (Griffin et al. 2000c). The usefulness of this marker in serum has not been demonstrated in humans.

Susceptibility

No general statements can be made about the susceptibility of rodent and non-rodent species to trichloroethylene compared with that in humans. It is important to evaluate potential mechanisms of immunotoxicity to determine whether those mechanisms are operative in humans. Pharmacokinetic modeling of specific metabolites of trichloroethylene is important to consider and apply to specific mechanisms that may be responsible for immunotoxicity.

Little is known about the genes that determine susceptibility to autoimmune and other immune diseases in humans. Although it is likely that environmental xenobiotics can act as triggers or exacerbants of autoimmune disease, there have not been adequate studies to make strong correlations. In addition, it is likely that multiple genes control susceptibility, some of which may play more important roles than others. There may be significant differences between rodent species and humans. It is important to explore genetic susceptibilities in human and animal models. Genes controlling metabolism and pharmacokinetic behavior of trichloroethylene likely are polymorphic and may influence effects on the immune system.

FINDINGS AND RECOMMENDATIONS

Laboratory results consistently show that some strains of mice are sensitive to autoimmune disease induction or exacerbation after exposure to trichloroethylene. The dose of

trichloroethylene required to produce effects depends on the route and length of exposure. It is difficult to extrapolate the results from genetically prone mice to humans, but these results suggest that humans with genetic susceptibilities may be at increased risk for autoimmune disease after exposure to by trichloroethylene. Animal data support the concept that trichloroethylene-mediated exacerbation of autoimmunity may be due to metabolism of trichloroethylene to chloral, which is a biologically plausible mechanism for humans.

Recommendations: More animal research is needed to clarify the metabolites and modes of action responsible for trichloroethylene-induced autoimmunity and immunosuppression. Epidemiology studies should further examine connective tissue diseases and other autoimmune diseases (including Stevens-Johnson Syndrome) or immunologic alterations (e.g., changes in T cell subsets, incidence of autoantibodies) in populations exposed to trichloroethylene.

Results from mouse studies suggest that chloral forms protein adducts that lead to an alteration of self proteins and the production of autoantibodies. CYP2E1, a known human polymorphic enzyme, may play a role in the formation of these protein adducts (Griffin 2000c). Inhibition of CYP2E1 was found to decrease the incidence and severity of autoimmune diseases in mice. Therefore, genetic polymorphisms in CYP2E1 may play a role in exacerbating autoimmune disease.

Recommendation: Genetic polymorphisms that may play a role in the metabolism of trichloroethylene should be further examined to determine sensitivity factors and to characterize potentially sensitive populations.

9

Special Populations and Susceptibility

The goal of the U.S. Environmental Protection Agency's (EPA) public health risk assessments is to protect all potentially affected populations, including subpopulations on the basis of gender, nutritional status, genetic predisposition, and life stages (e.g., childhood, pregnancy, old age) that might be more susceptible to toxic effects or that are highly or disproportionately exposed (e.g., children, ethnic groups) (EPA 2004). Children have been identified as a special population to consider in risk assessment because their health risks can differ from those of adults as a result of their immature physiology, metabolism, and different levels of exposure (EPA 1996, 2005b). Certain childhood cancers have been associated with exposures to solvents, including trichloroethylene, which is briefly discussed in this chapter.

Data on differential impacts to sensitive populations are frequently limited or absent, and the process for consideration of sensitive groups is poorly defined. As EPA stated, there is "not a single or exact method for examining potential susceptibility and associated risk" (EPA 2004, p. 43). In its draft risk assessment on trichloroethylene, EPA (2001b) relied on the evaluation by Pastino et al. (2000), who correctly stated that measures of susceptibility have not been incorporated into human risk assessment methods. Several papers have since been published that are relevant to this issue, particularly to pediatric risk and genetic susceptibility. These papers as well as relevant older studies and information on gender- and disease-based susceptibility are reviewed below.

CHILDHOOD CANCER

A sizable number of published scientific studies are relevant to the issue of parental occupational exposure to trichloroethylene and childhood cancer. The studies generally involve parental occupational exposures based on case-control studies. The committee did not have the time or resources to analyze all the studies, so it relied on a review paper (Colt and Blair 1998) and some new studies to illustrate the issues important in estimating the public health risk of parental exposure to occupational trichloroethylene and risk conveyed to their children. Chapter 2 provides guidelines for conducting a more rigorous review of the epidemiologic data.

The effects of parental occupational exposure on the risk of childhood cancer have been studied epidemiologically for more than 25 years. During that time, in most countries the nature of industry has changed in two important ways: through materials usage and levels of exposure. Specifically, trichloroethylene has been largely phased out as an industrial solvent. It is important to keep this in mind when reviewing the studies and their chronology. Colt and Blair (1998) reviewed information on parental exposure to solvents and the risk of childhood cancer. They summarized results from 48 published papers, virtually all of which were case-control studies. A few later papers have not clarified whether a relation exists (Shu et al. 1999; Schuz et al. 2000; McKinney et al. 2003; Infante-Rivard et al. 2005).

All studies used the case-control approach and therefore relied on questionnaire information, raising the usual methodological issues of the reliability of identifying specific exposures, selection of controls, and recall bias. Most studies relied on information about occupation and industry to infer exposures rather than questioning subjects about exposure to specific chemicals. Ages of the children studied varied from study to study. Often, only the mother was interviewed and asked about both her and her husband's occupational history. It is very unlikely that women know about specific occupational exposures of their husbands, which is illustrated in two studies by Peters et al. (1981, 1985). In the first study, excess childhood brain cancer was associated with maternal and paternal exposure to chemicals, specifically to paint, and to work in the aerospace industry. An open-ended question addressed to the mother about specific chemicals to which either parent was exposed revealed little for the mothers and two mentions of trichloroethylene for the fathers. A follow-up study in which the fathers were interviewed revealed that five of them had exposure to trichloroethylene, whereas no control fathers did. Much like the community studies, the same occupations had other exposures to materials such as methylethylketone and other unspecified solvents. Following the approach of asking both parents about exposure to specific chemicals, Lowengart et al. (1987), in studying childhood leukemia, showed a risk associated with paternal exposure to chlorinated hydrocarbons (most of which was trichloroethylene). Greater use was associated with higher risk. The previously cited study (Shu et al. 1999), using similar techniques in a larger study population, failed to show a risk associated with paternal exposures to trichloroethylene but the study period was considerably later when trichloroethylene was less likely to be used and when exposures were likely lower.

Theoretically, there are several ways that risk can be conveyed from parent to child. When the mother is exposed, her child might be exposed in utero, through breast milk, from contaminated clothing of the mother, or from germinal effects. For paternal exposure, bringing contaminated clothing home is possible or direct germinal effects to males transmitted during reproduction are possible. The latter is the most likely for trichloroethylene, given supportive animal toxicologic data (see Chapter 5).

Early studies on paternal occupational exposures suggest that exposure to trichloroethylene conveys a risk of leukemia and brain cancer developing in the offspring. Later studies do not show that risk. Over the time period of these studies, trichloroethylene was phased out as an industrial solvent and exposures in work settings have generally been reduced. Thus, the occupational studies provide data that suggest a relationship between parental exposure to trichloroethylene and risk of childhood cancers. The difficulties of studying rare diseases and the inability to measure exposure objectively limit any certainty about causality.

DEVELOPMENTAL ISSUES

Fetal and Pediatric Risk Assessment Concepts

Chemical-specific toxicokinetic and mode-of-action information are essential for fetal and pediatric risk assessment. As stated by a previous committee of the National Research Council, complete data would include the following (NRC 2000, p. 3):

1. the chemical's toxicokinetics (i.e., its absorption, distribution, metabolism, and excretion) within the mother, fetus, and embryo;
2. the chemical's toxicodynamics (i.e., how the chemical or a metabolite derived from it interacts with specific molecular components of developmental processes in the embryo and fetus or with maternal or extraembryonic components of processes supporting development);
3. the consequences of those interactions on cellular or developmental processes (also part of toxicodynamics); and
4. the consequence of the altered process for a developmental outcome, namely, the generation of a defect.

A framework evaluating developmental risk is similar to adult risk assessment in that it includes exposure assessment, toxicity assessment, and risk characterization. Each of these items involves evaluation of multiple parameters (Figure 9-1).

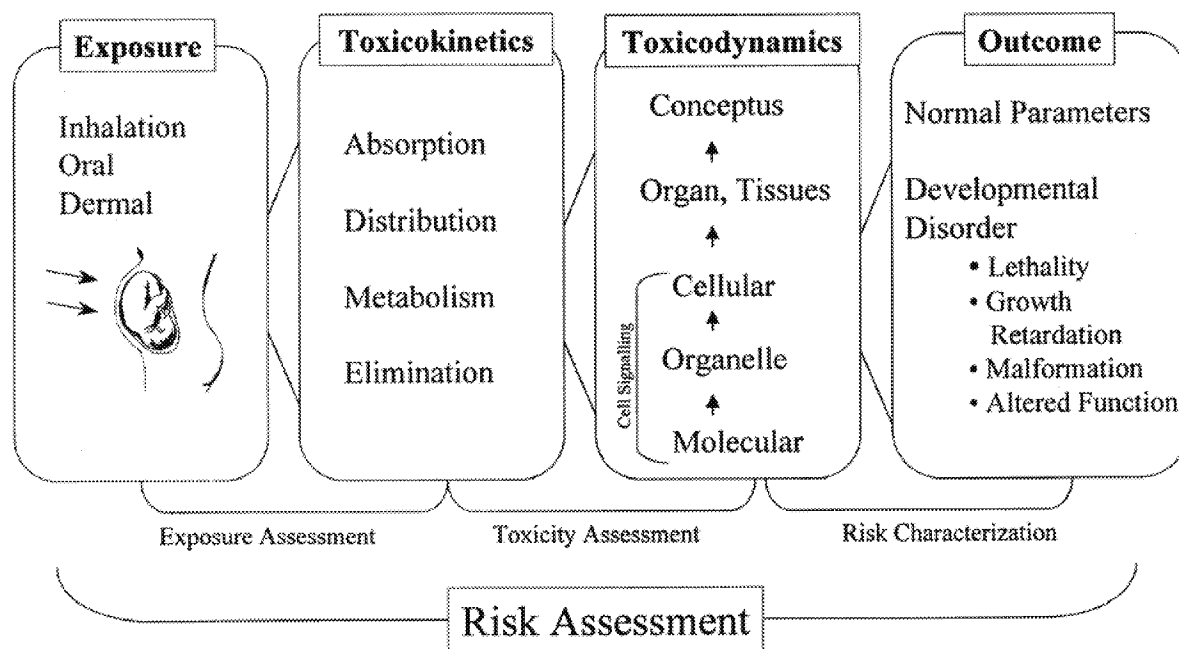


FIGURE 9-1 Overall framework to describe assessment of the effects of a toxicant on development.

Source: NRC 2000.

Tremendous advances have been made in recent years that facilitate the assembly of developmental toxicokinetic models. Such data include relevant ontogenic information about many of the phase I and phase II enzymes (Hines and McCarver 2002; McCarver and Hines 2002), which should be used for risk assessment (Dorne 2004). For the assessment of trichloroethylene, substantial data are available on toxicokinetics. Some, albeit less, information is available for items regarding developmental processes and their consequences (see Chapter 5). These advances, although still incomplete, can improve the precision of the risk assessment for trichloroethylene by narrowing uncertainty.

Approaches to Developmental Risk Assessment

Approaches defined under the new EPA guidelines for adult cancer risk assessment includes flexibility and the use of biologically based response models when appropriate (EPA 2005a). Similar flexibility and appropriate use of models are merited in evaluating toxicant risk for children under the ethical concept that children deserve, at a minimum, the same level of protection as adults. For any specific chemical, developmental susceptibility to toxicants could be addressed in several ways depending on the information available. These include the following:

1. Using developmental physiologically based pharmacokinetic (PBPK) modeling with parameter estimates appropriate for children: Such models can improve the development of relative-risk information for a specific xenobiotic exposure (Ginsberg et al. 2004a). The PBPK approach has already been used to evaluate pediatric drug therapy (Ginsberg et al. 2004b) and to assess adult risks from trichloroethylene (see Chapter 10); a similar effort should be applied for risk assessment in children. To facilitate such modeling, relevant data sets of developmental physiologic variables have been assembled and published (Haddad et al. 2001). A review of developmental pharmacokinetic modeling suggests that the 3.16-fold default uncertainty value commonly used for interindividual pharmacokinetic variability might be insufficient for some chemicals (Fawer et al. 1979). In contrast, Pelekis et al. (2001) developed a pediatric PBPK model for multiple volatile organic compounds using parameter estimates for a 10-kg child (1-2 years old) that suggests such children might not need additional protection. However, it is important to recognize that their model has not been validated with any in vivo developmental data; potentially this could be done with observational data. In addition, the model addresses only the parent compound and not the fractional metabolic clearance of any putative toxic metabolites; it is also a single-age model that does not reflect every period of childhood. For example, toddlers typically have enhanced clearance compared with younger and older children, as well as adults, because of enhanced relative liver and kidney size. Thus, although this is a good start in utilizing the PBPK approach, additional work should be done to ensure that the observation is robust and to assess the degree to which it applies across development. As stated by Ginsberg et al. (2004a), multiple age-appropriate physiologic models are needed. Because available information about the ontogeny of pathways involved in trichloroethylene disposition is substantial (see below), this approach would markedly decrease uncertainty about trichloroethylene exposures for the fetus and the child. However, similar to adults, this approach will not assess toxicodynamic differences and, importantly, will not address end points that might be unique to the fetus and the child. To be optimal, the information must be further

integrated using a serial approach that considers exposures serially from in utero to adulthood. Regardless of the limitations, this approach will significantly improve precision in risk estimates for children.

2. Using a developmental uncertainty factor: Such a factor could be added to the adult analysis to provide an arbitrary measure of added safety in consideration of a potential differential developmental susceptibility. This empiric approach has been used for risk assessment for childhood susceptibility for multiple chemicals. However, the approach should be used with decreasing frequency or the precision of the uncertainty factors should improve as more data become available. For trichloroethylene, insufficient toxicodynamic data necessitates using an uncertainty factor for toxicodynamic effects, but the uncertainty of increased susceptibility from exposure might be eliminated by using developmental PBPK modeling.

3. Establishing that children do not need greater protection than adults: If the variability present in adults is such that a sufficient margin of safety is present, an empiric decision could be made that no additional protection is needed for children. Generally, in risk assessment, this strategy is deemed appropriate when the variability is small and any estimate that ignores it will not be far from the truth. For developmental risk assessment, such a decision requires substantial justification, including detailed documentation of no added toxicodynamic risk during development. For trichloroethylene, as well as most other compounds, insufficient information is available to justify this approach.

Human Disposition of Trichloroethylene

EPA has clearly recognized the wide variability in trichloroethylene disposition, assigning a 50-fold variation, and also correctly recognized that this variability might contribute to susceptibility. Among adults, trichloroethylene disposition varies at least 7-fold, and perhaps as much as 50-fold (Lipscomb et al. 1997; Fisher et al. 1998). Variation in all aspects of trichloroethylene disposition—including absorption, distribution, metabolism, and excretion—affects the degree of biologic exposure to trichloroethylene and its metabolites (Astrand 1975). For example, trichloroethylene is poorly water soluble and highly fat soluble. After the same dose, trichloroethylene blood concentrations and urinary excretion of metabolites are expected to be greater in obese than in slim individuals (Sato et al. 1991). Similarly, the blood concentration of trichloroethylene and the total trichloroethylene body burden are expected to be higher in women than in men (Sato et al. 1991). Physical exertion during exposure to trichloroethylene, which is associated with increased pulmonary ventilation and cardiac output, is associated with increased adsorption, blood concentrations, and metabolite excretion (Astrand 1975).

Trichloroethylene is metabolized to chloral, which spontaneously hydrates to form chloral hydrate (Byington and Leibman 1965). This initial metabolic step, which might involve the transient formation of an intermediate epoxide (Miller and Guengerich 1982), is rate limiting and is catalyzed predominantly by the cytochrome P-450 enzyme CYP2E1 (Ikeda et al. 1980; Nakajima et al. 1992a; Lipscomb et al. 1997). Of multiple cytochrome P-450 enzymes tested in vitro, trichloroethylene metabolism to chloral hydrate correlated only with immunologically detected CYP2E1, and chloral hydrate formation significantly correlated with the oxidation of known CYP2E1 substrates (Lipscomb et al. 1997). The metabolism of chloral hydrate depends on two forward pathways (oxidation to trichloroacetic acid and reduction to trichloroethanol) and one back reaction (forming chloral hydrate from trichloroethanol). The reduction of chloral

hydrate to trichloroethanol is catalyzed by alcohol dehydrogenase (Friedman and Cooper 1960) and is NADH dependent, whereas the oxidation of chloral hydrate to trichloroacetic acid is mediated by aldehyde dehydrogenase (Cooper and Friedman 1958). The back reaction forming chloral hydrate from trichloroethanol appears to be catalyzed by CYP2E1 (Barton et al. 1996).

Trichloroethanol is also conjugated with glucuronate and the resulting water-soluble conjugate is excreted in the urine. Whether dichloroacetic acid is formed is controversial, with some investigators (Henderson et al. 1997) but not others documenting its presence in very low amounts in the blood of children given chloral hydrate for therapeutic indications. Brashear et al. (1997) found dichloroacetic acid at the limits of detection for mass spectrometry in adults exposed to trichloroethylene at 100 parts per million. Others have suggested that the presence of dichloroacetic acid is an analytic artifact and that dichloroacetic acid, if formed, is rapidly removed, precluding its measurement (Merdink et al. 1998). Further, no mechanism of dichloroacetic acid formation in humans has been described. The alternative to the initial CYP2E1-mediated trichloroethylene oxidation to chloral hydrate is direct conjugation of trichloroethylene with glutathione. This pathway accounts for less than 1% of the disposition of trichloroethylene and ultimately leads to the formation of cysteine conjugates or mercapturates (Green et al. 1997a). Although quantitatively a minor pathway, these metabolites have been associated with renal carcinogenesis and therefore are important in risk assessment (see Chapter 3).

Although intersubject differences in adsorption and distribution occur among adults, most of the variation in trichloroethylene disposition is secondary to differences in metabolism. In microsomal preparations from 23 human livers, CYP2E1-mediated trichloroethylene intrinsic clearance (V_{\max}/K_m) to chloral hydrate varied about 6-fold (Lipscomb et al. 1997). Among healthy volunteers exposed to ambient trichloroethylene, the urinary excretion of trichloroacetic acid and trichloroethanol varied 6- to 7-fold (Fisher et al. 1998), whereas trichloroacetic acid formation after dosing with chloral hydrate varied almost 10-fold, with 5% to 47% of the dose excreted as urinary trichloroacetic acid (Marshall and Owens 1954). In contrast to this striking between-subject variability, within subject variability appeared to be less than 2-fold.

Developmental Toxicokinetic Information

Improved analysis of trichloroethylene developmental toxicokinetics is important because recent population studies suggest that 3% to 7% of children have measurable amounts of trichloroethylene in their blood (Sexton et al. 2005). Although less is known about trichloroethylene disposition in children than in adults, substantial information is available that merits consideration in risk analysis. This includes information about the developmental profiles of CYP2E1, alcohol dehydrogenase, and aldehyde dehydrogenase, as well as studies of the disposition of chloral hydrate in children. The developmental profile of CYP2E1, the enzyme responsible for the rate-limiting step, has been well characterized across fetal and pediatric age groups (Johnsrud et al. 2003). Further, substantial pharmacokinetic information is known about the disposition of the major proximate metabolite, chloral hydrate, a sedative commonly used in children. Dichloroacetic acid, which may or may not be a metabolite in humans, is used therapeutically in the relatively rare disorder congenital lactic acidosis, and some pharmacokinetic data are available in adults and children, so that age-related comparisons are possible.

Important Physiologic Changes

Many physiologic changes directly affect developmental toxicokinetics, whereas others constitute toxicodynamic differences or additional toxicodynamic targets not present in adults. The biologically relevant internal dose of trichloroethylene as well as that of its metabolites likely is altered by multiple physiologic developmental changes. These include not only overall growth but also changes in body composition, relative organ size, and hormonal changes. Of particular importance to trichloroethylene and other compounds that deposit into fat is the doubling of body fat during early infancy with a concomitant fall in the amount of total body water. The liver and kidney, the predominant organs of overall toxicant activation and deactivation, are severalfold larger relative to body weight in children than in adults; this effect is greatest among toddlers (Maxwell 1984). Growth hormone concentrations increase during the newborn period and again with puberty (Quattrin et al. 1990; Rose et al. 1991; Main et al. 1994). Such growth hormone changes are associated with differences in drug disposition and drug-metabolizing enzyme expression (Redmond et al. 1978; Lambert et al. 1986; Butler et al. 1989). Growth hormone changes are associated with alterations in developmental gene expression mediated by many pathways, including the early response genes *c-fos* and *c-jun*, and HNF-6, a hepatic transcription factor that activates a network involved in cytochrome P-450 and plasma protein gene regulation (Rastegar et al. 2000). In addition to the differences in disposition that occur from growth and development, the fact that growth and development are occurring provides additional targets for derangement that are not present in adults. Thus, there are striking physiologic differences in children that affect xenobiotic disposition and likely alter their risk.

Developmental differences have been demonstrated in every aspect of pharmacokinetics; including absorption, distribution, metabolism, and excretion, using various therapeutic agents (see review by McCarver 2004). The impact of these differences on toxicokinetics has been less well evaluated. However, as stated above, *in vitro* and *in vivo* data have been generated that might facilitate PBPK modeling of trichloroethylene exposure in children. Oral and percutaneous absorption differences have been documented with many therapeutic agents (Heimann 1981; West et al. 1981; Rutter 1987; Barrett and Rutter 1994). Both routes are relevant for trichloroethylene exposure. Neither has been studied specifically for trichloroethylene; however, information generated for other compounds could be used to model the expected differences from these factors. Developmental variation in oral absorption is most marked in infancy and is due to differences in gastric pH, gastric emptying, pancreatic enzymes, and first-pass metabolism in the stomach, small intestine, and liver. Developmental differences in percutaneous absorption are due to differences in skin thickness, vascularization, and hydration. Distribution differs with age because of changes in body composition, protein, and tissue binding (Heimann 1981; Fisher et al. 1982; Nau et al. 1983; Lerman et al. 1989). An important distribution issue for the fetus is the ability of compounds to redistribute from amniotic fluid back to the fetus. In a rodent model, trichloroacetic acid was found to cycle from the fetus into the amniotic fluid and back into the fetus (Ghantous et al. 1986). Thus, amniotic fluid could act as a reservoir. Tissue drug binding, a more direct marker of the pharmacokinetic-pharmacodynamic interface than plasma values, might also be age dependent for some compounds (Park et al. 1982). Although the distribution of drugs across membranes recently has been shown to be influenced by a growing number of drug transporters, study of their ontogeny has just begun. It appears unlikely that a small, lipophilic, highly diffusible compound such as

trichloroethylene would require a transporter, but this issue has not been addressed. In summary, although these processes are complex, sufficient information exists to support exploration of PBPK modeling for trichloroethylene pediatric exposure.

Ontogeny of Human Enzymes Involved in Trichloroethylene Disposition

The use of generalities about the direction and extent of differences in metabolism in children compared with adults in risk assessment cannot be supported by currently available data. The developmental expression pattern varies by enzyme (Hines and McCarver 2002; McCarver and Hines 2002). Moreover, human and animal data are likely to differ, and such interspecies differences could be substantive. In contrast to rodents, human CYP isoform expression occurs relatively early, generally before birth or within the first several months of life (Hines and McCarver 2002). CYP2E1, the enzyme responsible for the rate-limiting step in trichloroethylene disposition, is expressed by week 8 of gestation in human fetal cephalic tissue at greater concentrations than in corresponding human fetal hepatic tissue (Brzezinski et al. 1999). In humans, hepatic expression occurs as early as the second trimester and rapidly increases after birth, with adult expression levels being reached by 3 months (Carpenter et al. 1996; Johnsrud et al. 2003). In contrast, rodent hepatic CYP2E1 expression begins postnatally (Keeter et al. 1990). Importantly, human CYP2E1 developmental expression data are sufficiently complete to support developmental PBPK modeling.

Differences in human alcohol dehydrogenase might be of equal or greater relevance to human trichloroethylene risk assessment compared with CYP2E1. Although CYP2E1 is the rate-limiting enzyme, the halogenated acetic acids trichloroacetic acid and dichloroacetic acid have been suggested as the proximate putative teratogenic species (Johnson et al. 1998a) as well as, perhaps, proximate nonrenal carcinogens (Herren-Freund et al. 1987; DeAngelo et al. 1989). As such, variability in alcohol dehydrogenase, as well as aldehyde dehydrogenase variability (discussed below), would influence the amount of chloral hydrate that is diverted to trichloroacetic acid, one of the putative toxicants. If alcohol-dehydrogenase-mediated conversion to trichloroethanol, a theoretically less toxic metabolite, is limited from immaturity or genetic factors, then more chloral hydrate would be available for conversion to trichloroacetic acid. However, this has not been directly confirmed in humans. Human alcohol dehydrogenase is a superfamily consisting of at least five classes encoded at seven genetic loci. Class I alcohol dehydrogenase includes three isoforms (α , β , and γ), and dimers of this class are the most effective at metabolizing ethanol to acetaldehyde. Thus, these isoforms have been well studied with ethanol (see below). In contrast, the relative capability of the various alcohol dehydrogenase isoforms in reducing chloral hydrate to trichloroethanol has not been published. As a general rule, for class I alcohol dehydrogenase, alcohols with bulky substituents are better substrates than ethanol. The ontogeny of alcohol dehydrogenase was described more than 30 years ago in seminal work by Smith et al. (1971), which was replicated and expanded by Estonius et al. (1996). In late gestation, fetal alcohol dehydrogenase activity is about 25% that of adult activity (Pikkarainen and Raiha 1967). Although expression is greatest in liver, class I alcohol dehydrogenase transcripts are widely distributed in all organs except fetal and adult brain, adult kidney, and placenta. In first-trimester human fetal liver samples, alcohol dehydrogenase α , encoded at *ADH1A*, is the only detectable class I isoform. Beginning in the second trimester and continuing into the early third trimester, hepatic alcohol dehydrogenases α ,

β , and γ (encoded by *ADH1A*, *ADH1B*, and *ADH1C*, respectively) are all present, but alcohol dehydrogenases α and β predominate. By the late third trimester, human hepatic *ADH1C* expression has increased markedly, but *ADH1B* expression still predominates. In adult liver, *ADH1A* expression is not detected, and expression of *ADH1B* and *ADH1C* is equivalent. In lung, only *ADH1B* expression is detected, and it is similar in adult and fetal samples (Estonius et al. 1996). Alcohol dehydrogenase class III is expressed in virtually all tissues, including in the fetus; expression in fetal brain appears to be somewhat greater than in adult brain.

Aldehyde dehydrogenase is a superfamily of NAD(P)⁺-dependent enzymes whose characteristics and substrate specificity vary (Vasilidou et al. 2004). The aldehyde dehydrogenase isoforms are encoded at 17 genetic loci (at least), and the various forms are highly expressed in the microsomal, mitochondrial, and soluble fractions of the liver as well as at lower levels in other tissues (Koivula 1975). Which aldehyde dehydrogenase isoforms are capable of and most efficient at metabolizing chloral hydrate to trichloroacetic acid is unknown but needs to be determined. *ALDH1A1*, *ALDH1A2*, *ALDH1A3*, and *ALDH8A1* are involved in the oxidation of retinaldehyde to retinoic acid, a critical factor in many developmental processes and signaling events. *ALDH1A1*, *ALDH1B1*, and *ALDH2* are all involved in the oxidation of acetaldehyde, the proximate metabolite of ethanol. In addition, *ALDH1L1* hydrolyzes 10-formyltetrahydrofolate to tetrahydrofolate, another reaction critical to development (Krupenko et al. 1997). Multiple forms of aldehyde dehydrogenase participate in the metabolism of 4-hydroxynonenal and malondialdehyde, two predominant products of human lipid peroxidation, and others in the formation of glutamate and in γ -aminobutyric acid metabolism. Treating rats with trichloroethylene depressed aldehyde dehydrogenase activity for short-chain aliphatic aldehydes in the mitochondrial and cytosolic fractions but not in the microsomal fraction (Wang et al. 1999), and this activity appears to be due to chloral hydrate [median inhibitory concentration = 8 μ M] (Wang et al. 1999; Poon et al. 2002). Thus, human aldehyde dehydrogenase theoretically is an important interaction point between trichloroethylene and multiple xenobiotics as well as multiple physiologic mechanisms. As such, more information on its role in trichloroethylene and chloral hydrate metabolism is needed.

Glutathione *S*-transferases (GSTs) are members of a family of enzymes that conjugate glutathione to electrophilic compounds. However, as noted above, glutathione conjugation represents the initial enzyme in the toxification pathway associated with renal carcinogenesis. The enzyme consists of two homodimeric subunits from one of five GST subunit classes (α , β , μ , π , θ , and ζ), and each enzyme is designated with a letter indicating its class membership (A, B, M, P, T, and Z, respectively). Which GST is most efficient in conjugating trichloroethylene is unknown. GST ontogeny was recently reviewed (McCarver and Hines 2002; Ginsberg et al. 2004a). Briefly, the hepatic α isoforms, *GSTA1* and *GSTA2*, are detected in the early fetal period and reach adult levels in the first 1-2 years of life (Strange et al. 1989). Fetal hepatic μ isoform expression is about 22% of adult expression and increases about 5-fold shortly after birth to adult levels (Strange et al. 1989). Isoform π is the predominant class in early hepatic development, being expressed in the human fetus and young infant at levels that exceed adult concentrations by 500- and 200-fold, respectively (Strange et al. 1989). *GSTA1* and *GSTA2* are also expressed in the fetal kidney and renal expression increases in the first two postnatal years, whereas *GSTM* is lower in postnatal than in fetal samples (Beckett et al. 1990). *GSTP1* has also been documented in early fetal renal collecting ducts (van Lieshout et al. 1998). Pulmonary developmental expression has also been evaluated with immunohistochemical and radioimmunoassays (Hiley et al. 1989). *GSTP1* is expressed at high concentrations in early fetal

pulmonary ductal columnar cells, but this expression decreases with gestation. In contrast, pulmonary expression of *GSTM*, *GSTA1*, and *GSTA2* are relatively low, but consistent, across gestation.

Chloral Hydrate and Dichloroacetic Acid Studies in Children

The trichloroethylene metabolite chloral hydrate has been used as a sedative for more than 150 years and is prescribed frequently for children, usually as a single dose for procedural sedation. Adults are typically given 1 g, whereas children are given 50-75 mg/kg up to the adult dose. The sedative effect is believed to be due to the metabolite trichloroethanol (Marshall and Owens 1954), although this assumption has been controversial (Mayers et al. 1992). Concern about chloral hydrate use in children includes its competition with bilirubin glucuronidation in the newborn period (Lambert et al. 1990) and the increased risk of arrhythmias, particularly among patients with congenital heart disease (Hirsch and Zauder 1986).

Concern about the possible carcinogenic potential of chloral hydrate in children (Steinberg 1993) resulted in a series of developmental studies by the National Toxicology Program (NTP 2002a,b). In a 2-year study, B6C3F₁ neonatal female mice under multiple chloral hydrate regimens, including groups given single or multiple doses for 2 years (both ranging from 0 to 100 mg/kg of body weight), the results were considered equivocal for carcinogenesis (NTP 2002a). The only positive result was an increased incidence of pituitary gland pars distalis adenomas at the highest dose (100 mg/kg for 24 months) without evidence of a dose response. Further, the incidence of pituitary adenomas in the high-dose group was similar to historical but not concurrent controls. No single-dose regimen was associated with carcinogenesis. In the 2-year B6C3F₁ male mouse study, increased incidence of hepatocellular adenoma or carcinoma (combined variable) was observed in mice fed ad libitum, and increased incidence of hepatocellular carcinoma was observed in dietary-controlled mice, which was associated with peroxisome proliferation (NTP 2002b). The concern over hepatocellular carcinogenesis was limited by the recognition that humans exhibited very weak liver peroxisome proliferative responses (Waxman 1999).

Although chloral hydrate is used clinically, the number of pharmacokinetic studies in children of various ages is limited. Critically ill infants and children (n = 22, ranging from 31 weeks postconceptional age to 13.6 years) participated in a pharmacokinetic study of chloral hydrate (Mayers et al. 1991). Patients were divided into three age groups, but it is unclear how many subjects were in each group. After a standard sedative oral dose (50 mg/kg, presumably to a maximum adult dose of 1 g), mean peak chloral hydrate plasma concentrations ranged from 3.89 ± 2.87 mg/L among toddlers and older children to 6.23 ± 2.28 mg/L among full-term infants to 8.01 ± 5.12 mg/mL among preterm newborns. After an initial rapid distribution phase, chloral hydrate terminal elimination occurred more slowly with a half-life of 1 to 10 hours. Apparent oral chloral hydrate clearance was about 5 L/hour/kg and did not differ by age. Trichloroethanol peak concentrations ranged from about 27 to 36 mg/L. Older children had a shorter trichloroethanol half-life of about 10 hours, whereas preterm and term infant groups had half-lives of about 40 and 28 hours, respectively. Because trichloroethylene is metabolized by glucuronidation, a pathway known to increase during the first few months of life, the observation of age-dependent trichloroethanol elimination was not surprising. Trichloroacetic acid increased to significant concentrations (10-20 mg/L) during the 7-day study and did not decline, so a half-

life could be determined only in one patient. The authors noted that the dose of metric area under the curve for trichloroacetic acid during the first 24 hours was greater among older children. They speculated that an inability of infants to form the oxidative metabolite trichloroacetic acid was responsible, which suggests that aldehyde dehydrogenase was immature in these subjects. Unfortunately, the study did not include urinary metabolite data, which are necessary for determining the relative conversion to trichloroethanol and to trichloroacetic acid. Henderson et al. (1997) described chloral hydrate kinetics in three children treated with chloral hydrate (50 mg/kg) alone; with chloral hydrate (50 mg/kg) 15 minutes before a dose of [^{13}C]dichloroacetic acid, or with two chloral hydrate doses before and after a dose of [^{13}C]dichloroacetic acid. Details of the gas chromatography-mass spectrometry assay including linearity and reproducibility were not given. Chloral hydrate peak values were not given. The peak concentration of trichloroethylene was 115 mg/mL at 25 minutes. Peak values for dichloroacetic acid (22 mg/mL) and trichloroacetic acid (65 mg/mL) occurred much later, at 7.5 and 11.5 hours, respectively. Importantly, the time course of trichloroacetic acid does not match that in other reports. Henderson et al. (1997) further reported coadministration of dichloroacetic acid with chloral hydrate prolonged the half-life of dichloroacetic acid, suggesting that chloral hydrate inhibits the metabolism of dichloroacetic acid. The pharmacokinetics of dichloroacetic acid have been determined in a few studies (Fox et al. 1996; Barshop et al. 2004), one of which included mostly children (Barshop et al. 2004). Among healthy adult volunteers (Curry et al. 1991), oral and intravenous dichloroacetic acid bioavailability were similar, and no gender difference was observed. The apparent terminal half-life was noted to increase with chronic dosing by more than 20-fold in healthy adults (Curry et al. 1985, 1991) and by more than 10-fold in lactic acidosis patients (Barshop et al. 2004). Among adults treated for lactic acidosis with single doses of dichloroacetic acid varying from 30 to 100 mg/kg, dichloroacetic acid elimination was zero order at concentrations above 80-120 $\mu\text{g/mL}$ and first order at lower concentrations with a terminal half-life of about 1.2 hours (Fox et al. 1996). The typical maximum concentration in both adult and pediatric lactic acidosis patients is 120-160 $\mu\text{g/mL}$ after 50 mg/kg intravenously (Fox et al. 1996; Barshop et al. 2004). Among 37 patients, including 31 children (ages 7 months to 17.8 years) treated with either single doses (50 mg/kg/day, $n = 6$) or repeated doses (variable doses, $n = 31$) for congenital lactic acidosis, the terminal half-lives were 86 minutes and 11 hours, respectively (Barshop et al. 2004). Although studies in healthy adult volunteers are relevant to the general population, metabolic studies in children and adults with lactic acidosis might not be relevant as the underlying acid-base abnormalities could alter enzymatic activity. Cord blood samples from 52 women given chloral hydrate during labor indicated that chloral hydrate crosses the human placenta (Bernstine et al. 1954). Chloral hydrate, trichloroethanol, and trichloroacetic acid were the same or higher in 50%, 63%, and 57% of cord blood samples, respectively, compared with paired maternal blood samples. Concentrations in amniotic fluid samples were described as matching fetal samples. In contrast to information for chloral hydrate, data on the *in vivo* human disposition of low concentrations of trichloroethylene in pregnant women have not been reported but are needed. The human ontogeny of both dichloroacetic acid and chloral hydrate metabolism *in vitro*, as well as chloral hydrate metabolism *in vivo*, merits further definitive study so that the data could be used for improved PBPK modeling.

GENETIC SUSCEPTIBILITY

Currently, 114 human CYP2E1 single nucleotide polymorphisms have been reported to the National Center for Biotechnology Information database dbSNP. Of these, 65 have been validated. Of the validated single nucleotide polymorphisms, 40 occur in introns, 3 occur in the mRNA untranslated region, and 22 occur in the coding sequence or in the upstream sequence. For many single nucleotide polymorphisms, the functional significance, if any, is unknown. Three allelic variants, *CYP2E1**2, *3, and *4 have been defined, each of which contains a single nucleotide polymorphisms leading to an amino acid change. However, two of these, *CYP2E1**3, consisting of a guanine to adenine substitution at nucleotide (10023 G>A) (leading to a valine to isoleucine change at amino acid 389 [V389I]), and *CYP2E1**4, consisting of 4768 G>A (leading to V179I), were associated with normal in vitro CYP2E1 activity (Hu et al. 1997; Fairbrother et al. 1998). In contrast, 1168 G>A causes an arginine to histidine substitution (R76H), which was associated with about a two-thirds decrease in both enzyme expression and activity in vitro (Hu et al. 1997). However, the variant is rare, occurring in about 1% of Asian populations and not detected among Europeans. Another single nucleotide polymorphism, adenine to thymine at nucleotide 11112 (11112 A>T), is associated with a histidine to leucine substitution (H457L), but has not been given a haplotype designation, and its functional significance has not been reported in vitro or in vivo (Solus et al. 2004). Two haplotypes, *CYP2E1**1B and *CYP2E1**1D, contain genetic polymorphisms in the upstream regulatory region. *CYP2E1**1D has been shown to be associated with increased CYP2E1 activity in vivo among individuals who are obese or who consume ethanol (McCarver et al. 1998). This insertion of 96 base pairs in the CYP2E1 regulatory regions occurs in about 25% of African Americans and a smaller percentage of Caucasians (McCarver et al. 1998); it represents the only known frequent, functional human CYP2E1 polymorphism described to date.

As noted above, it is unknown which human alcohol dehydrogenase isoform is most efficient at oxidizing chloral to trichloroethanol. The loci encoding two of the three class I alcohol dehydrogenase isoforms, *ADH1B* and *ADH1C*, exhibit functional genetic polymorphisms. Two *ADH1B* variants, *ADH1B**2 and *3, have been associated with decreased susceptibility to fetal alcohol spectrum disorders (McCarver et al. 1997; Viljoen et al. 2001; Das et al. 2004; Warren and Li 2005), presumably from increased ethanol elimination by the enzymes these variants encode (Thomasson et al. 1995; Neumark et al. 2004). Similarly, alcohol dehydrogenase genetic polymorphisms have been associated with differences in the risk for alcohol dependence (Thomasson et al. 1991) and cancer in some studies (Yokoyama et al. 2001; Yang et al. 2002; Coutelle et al. 2004) but not others (Olshan et al. 2001). The alcohol dehydrogenase variants are relatively common, occurring at allelic frequencies of 20% to 50%, depending on the ethnic group (Warren and Li 2005). Thus, it is plausible that the same alcohol dehydrogenase polymorphisms could be important factors for trichloroethylene teratogenic and carcinogenic susceptibility; studies directly addressing this hypothesis are needed.

Twenty-two aldehyde dehydrogenase genetic variants have been described, of which at least 11 appear to be functional (Vasiliou et al. 2004; www.aldh.org). Among the most studied variants is *ALDH2**2, which encodes a low-activity variant of mitochondrial aldehyde dehydrogenase and occurs in about 30% of individuals of Asian descent (Thomasson et al. 1991). This genetic variant is associated with a lower frequency of alcohol dependence and inability to oxidize acetaldehyde, yielding a flushing reaction during ethanol consumption (Itoh et al. 1997). Monte-Carlo analyses of the variability factors of other somewhat similar

compounds, such as ethanol and toluene, suggest that the default pharmacokinetic uncertainty factor is not sufficient to account for the *ALDH2**2 polymorphism (Ginsberg et al. 2002). The impact of aldehyde dehydrogenase variation on trichloroethylene disposition is unknown but is critical information for integrating genetic information into risk assessment.

GSTs are a family of phase II enzymes involved in the metabolism of many xenobiotics (Mannervik et al. 1992). Mammalian GSTs belong to three families of proteins that are expressed in cytosol, mitochondrial, and microsomal cellular fractions (Hayes et al. 2005). GSTs are catalytically active as hetero- or homodimers. These proteins are expressed in most human tissues, although numerous isoforms and subtypes are differentially expressed in cells and tissues (Strange et al. 1991). At least 16 cytosolic GSTs have been identified. These enzymes are named based on their amino acid sequences and immunologic characteristics (Board 1981; Mannervik 1985; Board et al. 1990). In general, mammalian cytosolic GSTs are divided into seven classes, which have been named alpha, mu, pi, sigma, theta, zeta, and omega. Many of the isoforms have known polymorphisms (Hayes et al. 2005). Examples include *GSTM1**A (associated with normal protein levels and activity), *GSTM1**B (associated with low protein levels), *GSTM1**0 (a gene deletion that leads to a null phenotype), and *GSTM1**1 × 2 (gene duplication). *GSTM1**0 is seen in more than 50% of some populations (Board et al. 1990). The *GSTT* family has at least three known polymorphisms (Strange et al. 1984). Various classes of GSTs, especially their null mutants, have been associated with cancer (Strange et al. 1991; van Poppel et al. 1992).

Whereas GSTs are generally considered to be important in the deactivation of electrophiles and oxidants, there are a few examples of bioactivation reactions. In these cases, the glutathione conjugate of a xenobiotic metabolite is more toxic than the xenobiotic or its metabolite alone. Such is the case for the glutathione conjugate of trichloroethylene known as *S*-1,2-dichlorovinyl-L-glutathione, which forms *S*-1,2-dichlorovinyl-L-cysteine in the presence of β -lyase (Dekant 1986). *S*-1,2-Dichlorovinyl-L-cysteine has been associated with kidney cancer (see Chapter 3), particularly in humans with a mutation in the von Hippel-Lindau gene. Populations with increased *GSTT1* activity and a *GSTM1* null allele have been found to be at particular risk for renal cell cancer (Bruning et al. 1997b).

Thus, depending on the specific GST isoform involved, a polymorphism in these enzymes can be expected to increase or decrease cancer risk. Predicting the human cancer or non-cancer risks in humans depends on the specific gene and polymorphism expressed. Data for trichloroethylene are incomplete; which GST isoforms are most efficient in trichloroethylene disposition is unknown.

ACQUIRED STATES WITH POSSIBLE ALTERED SUSCEPTIBILITY

Multiple conditions, including ethanol ingestion, exposure to other solvents, fasting or starvation, obesity and diabetes, and consumption of some popular dietary items, such as green tea, have been shown to induce CYP2E1 (McCarver et al. 1998; Lieber 2004; Liangpunsakul et al. 2005; Yang and Raner 2005). Many conditions associated with CYP2E1 induction are quite common and therefore likely to occur concomitantly with trichloroethylene exposure. Alcoholism affects about 14 million Americans annually; it is estimated that about half of Americans over the age of 12, or about 110 million Americans, consume ethanol (SAMHSA 2003). Thus, a clear understanding of the interactions between trichloroethylene and ethanol and

the effect on the trichloroethylene risk assessment is needed. The interaction between ethanol and trichloroethylene is complex and includes alterations in trichloroethylene kinetics and dynamics as well as those of its metabolites (for full discussion, see review by Pastino et al. [2000] and Chapter 10). Chronic heavy drinkers are likely to have enhanced trichloroethylene metabolism due to CYP2E1 induction. However, this is likely to be most relevant at high concentrations of trichloroethylene at which saturation would occur in the absence of enzyme induction. Chronic heavy drinkers who have progressed to cirrhotic liver damage could have decreased trichloroethylene metabolism. Individuals who have not recently consumed sufficient ethanol to induce CYP2E1 but who have acute, relatively concomitant exposure have decreased trichloroethylene metabolism from competitive inhibition (Muller et al. 1975). In addition to the effect on disposition, simultaneous exposure to ethanol and chloral hydrate increases the sedative effects, perhaps from the ethanol-mediated shift in chloral hydrate metabolism from oxidation to trichloroacetic acid to reduction to trichloroethanol (Watanabe et al. 1998). Obesity and diabetes induce CYP2E1 and both occur in millions of Americans (Harris 1995; Flegal et al. 2002). Although the impact of these common diseases would be expected to be similar to that from ethanol- or other solvent-mediated CYP2E1 induction, the relationships might be more complex. For example, increased body fat content affects trichloroethylene distribution so that urinary excretion rates are estimated to be greater in thin men than in obese men (Sato 1993). This effect might offset any enhanced CYP2E1-mediated metabolism in obesity. Notably, trichloroethylene-induced hepatotoxicity was not enhanced in a chemically induced rodent model of diabetes (Hanazono et al. 1975). Thus, direct study of the impact of these common states on trichloroethylene disposition and risk merits direct investigation.

GENDER

Simulated models suggest that, after the same dose of trichloroethylene, women have a slightly greater total body burden of trichloroethylene and its metabolites than men, reflected in a larger amount of total urinary metabolites (Sato et al. 1991). However, the size of this gender difference was relatively small (blood trichloroethylene concentrations 30% higher after 16 hours), and it was due to increased body fat content in women. In 16 volunteers (8 of each gender), equivalent ambient trichloroethylene exposure (100 parts per million for 4 hours) resulted in about 3.4-fold higher maximal concentrations and area-under-the-time-concentration curves of *S*-1,2-dichlorovinyl-*L*-glutathione in men than in women (Lash et al. 1999). This difference is intriguing because male rats were found to be efficient at trichloroethylene glutathione conjugation and male rat tubular cells were more susceptible to acute toxicity induced by *S*-1,2-dichlorovinyl-*L*-glutathione and *S*-1,2-dichlorovinyl-*L*-cysteine (Lash et al. 2001b). In contrast, one human case-control study of renal carcinogenesis showed an increased susceptibility for women (Dosemeci et al. 1999).

HUMAN VARIABILITY AND THE USE OF UNCERTAINTY FACTORS

Uncertainty factors are applied to risk estimates to account for variability in human populations as well as other factors. Following is a synopsis of EPA's use of uncertainty factors in its draft health risk assessment for trichloroethylene.

The oral reference dose for trichloroethylene non-cancer effects was calculated as 3×10^{-4} mg/kg-day, based on subchronic studies of rats and mice that showed effects at 1 mg/kg-day. The uncertainty factors included the following:

- A 50-fold uncertainty factor to account for differences between average and sensitive humans. This value was calculated by multiplying the value chosen for human pharmacokinetic variability (set at 15-20, see discussion below) by a $10^{1/2}$ -fold^a pharmacodynamic variation, which is the EPA default value.
- A $10^{1/2}$ -fold default uncertainty factor for animal-to-human pharmacodynamic uncertainty. The previous value (15-20) was considered to account for animal-to-human pharmacokinetic uncertainty.
- A $10^{1/2}$ -fold uncertainty for using subchronic instead of lifetime studies. EPA states that duration-response trends are not evident in animal studies, but some human studies indicate that prolonged exposure to trichloroethylene can increase the severity of effects. Therefore, the partial $10^{1/2}$ -fold uncertainty was used “until duration-response relationships are better characterized in humans.”
- A $10^{1/2}$ -fold uncertainty factor for extrapolation from a lowest-observed-adverse-effect level (LOAEL) to a no-observed-adverse-effect level (NOAEL) because adverse effects were observed at the 1 mg/kg-day point of departure. EPA stated that the standard 10-fold default was not used because “1 mg/kg-day appears to be at the boundary where effects can begin to be observed.”
- A $10^{1/2}$ -fold uncertainty factor to reflect background exposures to trichloroethylene and its metabolites to address cumulative risks.

In total, this generates a 5,000-fold uncertainty factor. Ultimately, however, a factor of 3,000 was used as the divisor because it is the largest divisor used by EPA in the presence of substantial uncertainty (EPA 2001b).

The trichloroethylene inhalation concentration of 4×10^{-2} mg/m³ was based on a subchronic exposure of 38 mg/m³ showing adverse effects on the central nervous system in human occupational studies. The uncertainty factors included the following:

- A 10-fold default uncertainty factor for human variation,
- A 10-fold default uncertainty factor for using subchronic instead of lifetime studies, and
- A 10-fold default uncertainty factor for extrapolation from a LOAEL to a NOAEL uncertainty because the central nervous system and endocrine effects were LOAELs observed in occupational studies.

For cancer end points, EPA chose to use a range of slope factors in view of risk factors that can modify the effects of trichloroethylene in different populations. Of the cancer studies evaluated (including rodent and human studies), the highest and lowest cancer slope factors were not used and the range of the remaining studies was maintained. According to EPA “these

^a $10^{1/2}$ (the square root of 10, approximately equal to 3) represents half a factor of 10 on a logarithmic scale. In this case, the $10^{1/2}$ factor was the default for the pharmacodynamic variation. In other instances (e.g., LOAEL to NOAEL extrapolation), the use of this lesser factor is supported by qualitative information presented in EPA’s discussion of the uncertainty factors.

remaining estimates constitute a middle range of risk estimates where confidence is greatest” (EPA 2001b). Recognizing differences in human responses and the potential for sensitive populations, EPA further states that “a single risk value is not appropriate to describe the differential effects of [trichloroethylene]” and “alternative slope factors have not been consolidated into a single estimate.”

For determining the oral reference dose, EPA used a method other than the default factor for considering human variability. Instead, a 15- to 20-fold factor was used based on the uncertainty of the potency for mouse liver tumor production. These uncertainty factors for potency were assumed to be the same as the uncertainty in the internal dose estimates defined as the area under the curve for trichloroacetic acid and dichloroacetic acid, respectively. This 15- to 20-fold factor was assumed to account for both human pharmacokinetic variability and the pharmacokinetic differences between animals and humans.^b The derivation of the 15- to 20-fold uncertainty factor was not thoroughly explained. EPA states: “A factor of 15-20 reflects the pharmacokinetic uncertainty in the liver between the 50th and 99th percentiles (see Table 9-1).” In the risk assessment, EPA supports this value with studies demonstrating that continuing exposure to trichloroethylene can increase the severity of effects. Somewhat paradoxically, the same studies were used to support the lesser 10^{1/2}- fold uncertainty factor for the oral dose.

Table 9-1, reproduced below, is adapted from Table 15 of Rhomberg (2000), which details the uncertainty in human risks of liver tumors based on an analysis of mouse liver tumors using trichloroacetic acid and dichloroacetic acid as an internal dose measure. The uncertainty for human potency in producing liver tumors is estimated by assuming the toxic equivalency of internal dose (presented as the area under the curve) between humans and mice. The uncertainty

TABLE 9-1 Approximate Uncertainty Analysis Based on Log-Normal Error

Human potency based on	Uncertainty in potencies										
	Uncertainty in animal internal dose, GSD _A				Uncertainty in human internal dose, GSD _H			Uncertainty in human potency dose, GSD _{POT}			
Mouse liver, TCA-auc	2.1				2.4			3.2			
Mouse liver, DCA-auc	2.7				2.2			3.6			
Rat kidney, thiol	3.4				6.2			9.0			
Mouse lung, CH-auc	3				9			11.7			
Mouse lung, CH-max	3.5				9			12.5			
	Factor different from median estimate										
	Percentile of potency uncertainty distribution										
Human potency based on	1	2.5	5	10	25	50	75	90	95	97.5	99
Mouse liver, TCA-auc	1/15	1/10	1/7	1/4.4	1/2.2	1	2.2	4.4	7	10	15
Mouse liver, DCA-auc	1/20	1/12	1/8	1/5	1/2.4	1	5	5	8	12	20
Rat kidney, thiol	1/170	1/74	1/37	1/17	1/4.4	1	4.4	17	37	74	170
Mouse lung, CH-auc	1/300	1/120	1/56	1/23	1/5.2	1	5.2	23	56	120	300
Mouse lung, CH-max	1/360	140	1/63	1/25	1/5.4	1	5.4	25	63	140	360

Abbreviations: CH, chloral hydrate; DCA, dichloroacetic acid; TCA, trichloroacetic acid.
Source: Rhomberg 2000. Reprinted with permission; copyright 2000, Environmental Health Perspectives.

^b From EPA (2001b, p. 4-7): Human variation: The NOAELs, LOAELs, and LED₁₀s for adverse liver effects were estimated using a pharmacokinetic model. The parameter uncertainty in these modeled dose estimates (estimated between the 50th and 99th percentiles, see Table 9-1) is 15-fold if plasma TCA [trichloroacetic acid] is used as the dose metric and 20-fold if plasma DCA [dichloroacetic acid] is used.

distributions for the animal internal dose and human internal dose—which were derived from a Bayesian uncertainty analysis (Bois 2000a) using the Clewell et al. (2000) model—were mathematically combined to estimate the distribution for the uncertainty in human potency. As mentioned, EPA used the fold difference between the 50th and 99th percentile to estimate both the variability in human pharmacokinetics and the uncertainty regarding the extrapolation of pharmacokinetic parameters from animals to humans. Problems with considering this as a measure of human pharmacokinetic variability include its derivation from mouse data, use of the Clewell model compared with others, and use of an assessment of the variability in cancer potency for assigning the variability of non-cancer effects.^c

FINDINGS AND RECOMMENDATIONS

The scientifically appropriate inclusion of human variability into risk assessment is an ongoing challenge. EPA has attempted to account for human variability, particularly for vulnerable populations, with an array of uncertainty factors. EPA is encouraged to increase the precision of risk estimates used for fetuses and children with PBPK modeling approaches similar to that used for adults. Similar approaches also can be used to account for ethanol consumption and exposure to compounds with known metabolic interactions. Multiple suggestions are given below for additional data analysis and data generation, particularly to advance understanding of the role of genetic polymorphisms in trichloroethylene disposition as determinants of susceptibility. The committee questions whether the use of variability in animals to approximate human variability is appropriate.

Increased use of PBPK modeling in developmental risk assessment is essential for addressing health issues specific to children. This approach would increase the precision of risk assessment by enhancing the understanding of biologically relevant dosimetry related to fetal or pediatric exposures compared with adult exposures as well as, in some cases, allow for extrapolations across routes of exposure. In addition, available animal studies with blood concentrations could be used to model relevant target tissue concentrations (e.g., central nervous system or kidney) that are necessary to cause specific end point effects with identified modes of action. If such modeled target tissue concentrations were available for both animals and humans, it would enhance the ability to determine whether a developmental risk is plausible based on relevant tissue dosimetry. Within the developmental PBPK modeling, it is important to recognize that children do not represent a single group and that several physiologic stages must be considered.

It is unknown which human enzymatic isoforms dispose of trichloroethylene and its metabolites most efficiently. This information is critical for determining the relevance of various common functional genetic polymorphisms already known among enzyme families involved in trichloroethylene disposition as well as those that might be identified in the near future. Knowing the relevance of these genetic polymorphisms to risk assessment could then be

^cWhile the risk assessment uses this uncertainty factor in the calculations for effects other than cancer, Rhomberg's analysis derives this uncertainty distribution for potency in producing liver tumors. In its analysis, EPA accepted the assumption that the uncertainty distribution of the internal dose at the lowest experimental exposure is a reasonable approximation of the uncertainty in low internal dose potency. However, the EPA assessment is not congruent with the fact that these uncertainty factors do not include estimates of human pharmacokinetic variation.

determined. Approaches include PBPK modeling such as that already performed for parathion and warfarin (Gentry et al. 2002).

PBPK models are needed, but do not address the well-recognized pharmacodynamic differences between children and adults. Intersubject variation in pharmacodynamic factors has not been well quantitated among adults and pharmacodynamic modeling of toxicant effects has not been performed. Further, modeling of long term end points of toxicant effects is difficult. For this to be attempted, the critical end points must be defined and appropriate shorter term effect biomarkers of these end points validated.

Recommendations: PBPK models for different physiologic stages of childhood development should be created for trichloroethylene. Research on children's exposure to trichloroethylene will be required to support model development, including measurement of trichloroethylene metabolites in breast milk and biological matrices from children (e.g., cord blood, amniotic fluid, and meconium) in different age groups.

- Improved information on dermal absorption and alterations in risk from developmental differences in skin thickness, as well as surface area and body weight determinations, is needed.

- If interspecies differences are determined to be predominantly related to compound disposition, PBPK models that incorporate critical comparative biology and physiology can be used to extrapolate developmental studies in animals to humans.

- More research is needed to understand which human enzymatic isoforms are most important in disposing trichloroethylene and its metabolites.

- Better characterization is needed of the impact of physiologic conditions and disease states on trichloroethylene toxicity, particularly with low-dose chronic exposure. It is possible that existing data sets could be mined for pertinent information, particularly for common disorders or factors, such as diabetes, obesity, and alcohol consumption.

- Additional data regarding intersubject variation in pharmacodynamic differences is needed across life stages and in various subpopulations before pharmacodynamic factors can be quantitated in risk assessment. Before such pharmacodynamic data can be generated, the critical targets and modes of action must be clarified from animal or in vitro studies.

10

Mixtures

Potential and known interactions between carcinogens and noncarcinogens in chemical mixtures in the environment have been a concern for several decades. Toxicokinetic and toxicodynamic interactions might result in decreased (antagonistic), exaggerated, additive, or unchanged toxicity relative to that of individual components. Although exaggerated toxicity is the primary concern, the antagonistic interaction resulting in decreased toxicity would also affect the assessment and management of risk.

Kidney and liver are the major target organs for trichloroethylene toxicity resulting from the generation of reactive metabolites through glutathione conjugation and cytochrome P-450-mediated metabolism. Human health risks of trichloroethylene stem mainly from its carcinogenic potential. In the body, trichloroethylene is metabolized into trichloroacetic acid, chloral hydrate, 2-chloroacetaldehyde, trichloroethanol, trichloroethanol glucuronide, and perhaps dichloroacetic acid. Because trichloroethylene is readily absorbed by all routes of exposure and extensively metabolized to multiple chemical species, exposure to trichloroethylene can be considered an exposure to a toxic mixture. Information on trichloroethylene metabolite toxicity is helpful in identifying the metabolites responsible for toxicity and might influence the effect of coexposures to other toxicants, particularly if they directly or indirectly change the proportions of trichloroethylene metabolites.

This chapter presents an overview of mixture toxicology, some of the important coexposure issues to consider in evaluating trichloroethylene, and possible approaches to using pharmacokinetic modeling for making predictions.

TOXICOLOGY OF MIXTURES CONTAINING TRICHLOROETHYLENE

Laboratory toxicity testing of single compounds can produce toxicity data specific to that compound for that species, but it cannot take into account the possible toxic effects of mixtures of compounds. For example, in a 6-month carcinogenicity assay, trichloroethylene-contaminated groundwater was found to be carcinogenic in Japanese medaka fish, after initiation with diethylnitrosamine (Gardner et al. 1998). Analysis of the groundwater indicated that contamination was not limited to trichloroethylene. No tumor promotional effect was found in a

follow-up laboratory study with reagent-grade trichloroethylene added to the groundwater to simulate the exposure concentration found in the contaminated groundwater. These studies implicate other water contaminants that might synergize the tumor-promoting activity of trichloroethylene.

Acute or repeated inhalation exposure to a mixture of 1,1,1-trichloroethane, 1,1-dichloroethane, trichloroethylene, and tetrachloroethylene at concentrations as low as 20 parts per million (ppm) produced neurologic impairment. Male and female weanling ICR mice were treated with a mixture of chlorinated alkanes and alkenes consisting of chloroform, 1,1-dichloroethane, 1,1-dichloroethylene, 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene in drinking water for 16 and 18 months, respectively; male mice developed hepatocellular neoplasms and female mice developed mammary adenocarcinoma (Wang et al. 2002). The toxicokinetics of trichloroethylene was altered in rats receiving a binary mixture of chloroform and trichloroethylene (Anand et al. 2005a). Metabolism of trichloroethylene is suppressed in humans with coexposure to tetrachloroethylene (Seiji et al. 1989). Exposure to a ternary mixture of chloroform, trichloroethylene, and allyl alcohol results in less initial liver injury in male Sprague-Dawley rats because of greater elimination of trichloroethylene (Anand et al. 2005b).

A number of commonly used drugs modify the metabolism of trichloroethylene (Leibman and McAllister 1967; Carlson 1974; Moslen et al. 1977; Pessayre et al. 1979). The opposite might also occur, resulting in important modifications of the therapeutic action of the drugs (Kelley and Brown 1974). Trichloroethylene competitively inhibits the metabolism of barbiturates, producing exaggerated effects of the drugs (Kelley and Brown 1974). Sellers and Koch-Weser (1970) observed a potentiation of the anticoagulant effect of bishydroxycoumarin (warfarin) in patients after chloral hydrate ingestion, which appears to result from displacement of plasma protein binding sites by the chloral hydrate metabolite trichloroacetic acid. Trichloroacetic acid is extensively bound to plasma proteins (Templin et al. 1995), making it likely that trichloroethylene might potentiate the effects of many other drugs that normally bind to the same protein sites. Ethanol (2 g in daily liquid diet for 3 weeks) pretreatments enhanced hepatic damage in male Wistar rats treated with trichloroethylene (inhalation exposures of 500 ppm for 8 hours, 2,000 ppm for 2 or 8 hours, and 8,000 ppm for 2 hours) (Okino et al. 1991). Chemical coexposures from the environment in addition to human behaviors, such as alcohol consumption, might have effects that overlap with hepatic damage in male Wistar rats in terms of toxicokinetics, pharmacodynamics, and target tissue toxicity (see also Chapters 9 and 11). Alcohol consumption is a common coexposure that has been noted to affect trichloroethylene toxicity (see discussion later in this chapter). Coexposure to trichloroethylene might increase the toxicity of methanol and ethanol by altering their metabolism to aldehydes and also by altering their detoxification. Concomitant administration of alcohol and chloral hydrate in humans exacerbated the side effects of chloral hydrate (e.g., vasodilation, tachycardia, hypotension) (Sellers et al. 1972; Muller et al. 1975). The intolerance syndrome resulting from combined exposure to trichloroethylene and ethanol is due to increased accumulation of trichloroethylene in the central nervous system resulting from depression of trichloroethylene oxidation. Therefore, there is adequate basis for interactions to modulate the toxicity of trichloroethylene upon coexposure to other chemicals.

Interaction of metals with trichloroethylene could result in altered absorption of the metals. Dermal penetration of nickel significantly increased when it was administered along with phenol, toluene, and trichloroethylene to dermatomed male pig skin samples in flow-

through diffusion cells. Consequently, the potential health risk from dermal exposure to nickel is enhanced if other chemicals are present (Turkall et al. 2003). Not all metals interact with trichloroethylene in the same way. When lead carbonate and trichloroethylene were given concurrently to male rats, no additive or synergistic neurotoxicities were observed (Nunes et al. 2001).

Coexposures to trichloroethylene, trichloroacetic acid, and dichloroacetic acid at environmental concentrations are not uncommon (Wu and Schaum 2000). Trichloroethylene and tetrachloroethylene share common metabolites that have similar actions and targets and, therefore, coexposures potentially increase the risk from exposure to trichloroethylene. Trichloroethylene and di-(2-ethylhexyl)phthalate, a peroxisome proliferator, were reported to synergize prenatal loss, cause a decrease in pup weight, and cause anaophthalmia in rats (Narotsky and Kavlock 1995; Narotsky et al. 1995).

Veeramachaneni et al. (2001) reported effects in male rabbits exposed to drinking water containing chemicals at concentrations typical of groundwater near hazardous waste sites (the exposure mixture contained arsenic, chromium, lead, benzene, chloroform, phenol, and trichloroethylene). Even at 45 weeks after last exposure to drinking water pollutants, mating desire or ability, sperm quality, and Leydig cell function were subnormal. However, although the exposure concentrations are relevant to human environmental exposures, the design of this study precludes a conclusion about what combination of the seven toxicants, or what individual toxicant, caused the effects, exemplifying the problems associated with studying toxicology of a multicomponent mixture. Recent literature on interactions of trichloroethylene metabolites and common coexposures report the interactions of two or three chemicals at a time and use several approaches including examination of tumor phenotype, gene expression, and development of physiologically based pharmacokinetic (PBPK) models to assess possible synergy, antagonism, and additivity of effects or toxicokinetics. These studies may provide insights into possible modes of action and modulators of trichloroethylene toxicity.

One area that still hampers the risk assessment is interindividual differences that lead to variation in toxic responses in human populations. Although many factors are involved and the science still does not allow us to quantitate the influence of each factor, little is known about the influence of diet and caloric intake on trichloroethylene toxicity. A diet rich in carbohydrates protects male Wistar rats from liver injury by decelerating the transformation of trichloroethylene to highly toxic intermediates (Nakajima et al. 1982). A combination of ethanol with a low-carbohydrate diet accelerates the metabolism and enhancement of hepatotoxicity of trichloroethylene in male Wistar rats (Sato et al. 1983). A dietary copper imbalance resulted in higher trichloroethylene-induced lung damage as evidenced by a larger number of vacuolated Clara cells (Giovanetti et al. 1998).

Some of the important coexposures that affect the toxicity of trichloroethylene are discussed below.

Contaminants of Trichloroethylene

Earlier carcinogenic studies (NCI 1976) with trichloroethylene were faulted because they used commercial grade trichloroethylene as the test agent, which could contain stabilizers such as epichlorohydrin, a known carcinogen. Henschler et al. (1984) studied the effects of oral administration of trichloroethylene with and without stabilizers (epichlorohydrin and 1,2-

epoxybutane) on ICR/Ha Swiss mice. They concluded that there was an increase in forestomach cancers in the mice treated with trichloroethylene containing stabilizers, but there was no effect on the induction of liver tumors. They attributed the increase in forestomach cancers to the direct alkylating properties of epichlorohydrin and epoxybutane.

Interactions Between Trichloroacetic Acid and Dichloroacetic Acid

A recent study (Bull et al. 2002) attempted to examine how coexposures and variations in relative concentration between two trichloroethylene metabolites, dichloroacetic acid and trichloroacetic acid, might affect toxicity. Bull et al. (2002) reported that the tumor phenotype in B6C3F₁ male mice depended on the proportion of the two chemicals administered after 52 weeks of exposure. Given alone, trichloroacetic acid (0.5 or 2 g/L) and dichloroacetic acid (0.1, 0.5, or 2 g/L) produced liver tumors in mice with phenotypic characteristics that are distinct in several respects, with each compound at doses that were not cytotoxic. Combinations of trichloroacetic acid (0.5 or 2 g/L) and dichloroacetic acid (0.1 or 0.5 g/L) resulted in dose-related increases in hepatic preneoplastic lesions, adenomas, and carcinomas greater than either compound alone and in an additive fashion, with the addition of dichloroacetic acid to fixed exposures to trichloroacetic acid causing an increase in adenomas but not in carcinomas. Given alone, dichloroacetic acid produces tumors in mice that display a diffuse immunoreactivity to a *c-Jun* antibody, whereas trichloroacetic acid-induced tumors do not stain with this antibody. When given in various combinations, dichloroacetic acid and trichloroacetic acid produced a few *c-Jun*⁺ tumors, and many that were *c-Jun*⁻, but a number with a mixed phenotype increased with the dose of dichloroacetic acid. A comparison of tumor phenotypes induced by trichloroethylene (1 g/kg) shows that such tumors also have a mixture of phenotypes, suggesting that trichloroethylene-induced tumors are not consistent with either trichloroacetic acid or dichloroacetic acid acting alone.

Coexposures to Other Haloacetates

Other haloacetates produced in the bromination of drinking water might affect trichloroethylene toxicity through a similarity of effects of its metabolites. Kato-Weinstein et al. (2001) reported that brominated haloacetates such as bromodichloroacetate, bromochloroacetate, and dibromoacetate appear at higher concentrations in drinking water than the chlorinated haloacetates dichloroacetic acid and trichloroacetic acid. To study the similarity in action between the brominated and chlorinated haloacetates, mice were administered dibromoacetate, bromochloroacetate, and bromodichloroacetate in drinking water at concentrations of 0.2-3 g/L for 12 weeks (Tao et al. 2005). The dihaloacetates, bromochloroacetate and dibromoacetate, caused liver glycogen accumulation similar to that of dichloroacetic acid. The authors noted possible contamination of bromochloroacetate with dichloroacetic acid and dibromoacetate in their studies. The trihaloacetates, trichloroacetic acid and low concentrations of bromodichloroacetate, produced slight decreases in liver glycogen content, especially in the centrilobular region. The high concentration of bromodichloroacetate produced a pattern of glycogen distribution similar to that in dichloroacetic acid-treated mice. All dihaloacetates reduced the amount of serum insulin at high concentrations. Conversely, trihaloacetates had no

significant effects on serum insulin concentrations. After up to 26 weeks of treatment, dibromoacetate was the only brominated haloacetate that consistently increased acyl-coenzyme A oxidase activity (a marker of peroxisome proliferator-activated receptor α) agonism and rates of cell replication in the liver, but these effects were limited to 2-4 weeks of treatment and at exposures > 1 g/L (Tao et al. 2004a).

Coexposures to Other Solvents

Promotional and gene expression effects of trichloroethylene metabolites have been investigated in a number of studies in which they were administered after initial treatment with other carcinogens. Bull et al. (2004) studied interactions of metabolites (trichloroacetic acid and dichloroacetic acid) and carbon tetrachloride, motivated by the fact that trichloroethylene and carbon tetrachloride are commonly found together at contaminated sites. B6C3F₁ male mice, initially treated vinyl carbamate (3 mg/kg) at 2 weeks of age, were treated with dichloroacetic acid (0.1, 0.5, or 2.0 g/L), trichloroacetic acid (0.1, 0.5, or 2.0 g/L), and carbon tetrachloride (50, 100, and 500 mg/kg, and then reduced at week 24 to 5, 20, and 50 mg/kg due to toxicity) or pairwise combinations of the three compounds for 18-36 weeks. Histopathologically, a sample of 100 lesions was examined to verify that the criteria for the general descriptor of neoplastic and nonneoplastic lesions were satisfied. As the dose of carbon tetrachloride increased, the number of tumors per animal increased, whereas mean tumor size decreased. When administered alone in drinking water, dichloroacetic acid increased both tumor number and tumor size in a dose-related manner. With trichloroacetic acid treatment, tumor numbers plateaued by 24 weeks at a high dose. Dichloroacetic acid treatment did not produce a plateau in tumor number within the experimental period, but the numbers observed at the end of the experimental period (36 weeks) were similar to those found with trichloroacetic acid and to doses of carbon tetrachloride at 50 mg/kg.

Differing combinations of the three agents in initiated animals gave more complex results between 24 and 36 weeks of observation. At 24 weeks, dichloroacetic acid produced a decrease in tumor numbers promoted by trichloroacetic acid, but the numbers were not different from those for trichloroacetic acid alone at 36 weeks. The reason for this result became apparent at 36 weeks of treatment, when dichloroacetic acid coadministration led to a dose-related decrease in the size of tumors promoted by trichloroacetic acid. However, the low dose of trichloroacetic acid decreased the number of tumors produced by a high dose of dichloroacetic acid (2 g/L), but higher doses of trichloroacetic acid (2 g/L) produced the same number that was observed with dichloroacetic acid alone. Dichloroacetic acid inhibited the growth rate of carbon tetrachloride-induced tumors. Trichloroacetic acid substantially increased the number of tumors observed at early time points when combined with carbon tetrachloride, but this effect was not observed at 36 weeks. The lack of an effect at 36 weeks was attributed to the fact that more than 90% of the livers consisted of tumors and the earlier effect was masked by coalescence of the tumors. Thus, trichloroacetic acid significantly increased tumor numbers in mice treated with carbon tetrachloride.

Pretreatment with trichloroethylene in drinking water at concentrations as low as 15 mM for 3 days has also been reported to increase susceptibility to liver damage to subsequent exposure to a single intraperitoneal injection (1 mL/kg) of carbon tetrachloride in Fischer 344 rats (Steup et al. 1991). Several mechanistic hypotheses offered included altered metabolism,

decreased hepatic repair capability, decreased detoxification ability, or a combination of these. Simultaneous administration of trichloroethylene (0.5 mL/kg) also increased the liver injury induced by carbon tetrachloride (0.05 mL/kg) (Steup et al. 1993). The authors suggested that trichloroethylene appeared to impair the regenerative activity in the liver, thus leading to increased damage when carbon tetrachloride is given in combination with trichloroethylene.

Chloroform, a chlorine disinfection by-product found in drinking water, as well as dichloroacetic acid and trichloroacetic acid, is also a mouse liver carcinogen and was the focus of another study by Pereira et al. (2001). They reported the effects of coexposure to chloroform (0, 400, 800, 1,600 mg/L) on hypomethylation and expression of the *c-myc* gene induced by treatment with dichloroacetic acid and trichloroacetic acid (500 mg/kg) in the livers of female B6C3F₁ mice. Dichloroacetic acid, trichloroacetic acid, and to a lesser extent chloroform decreased methylation of the *c-myc* gene. Coadministering chloroform (at 800 and 1,600 mg/L) decreased dichloroacetic acid-induced hypomethylation but it had no effect on that of trichloroacetic acid. Expression of *c-myc* mRNA was increased by dichloroacetic acid and trichloroacetic acid, with the two highest doses of chloroform attenuating the actions of dichloroacetic acid but not trichloroacetic acid.

In the same study, male and female B6C3F₁ mice, administered *N*-methyl-*N*-nitrosourea (an initiator of liver and kidney tumors) on day 15 of age, and dichloroacetic acid (3.2 g/L) or trichloroacetic acid (4.0 g/L) with chloroform (0, 800, or 1,600 mg/L) starting at 5 weeks of age, were examined after 36 weeks for promotion of liver and kidney tumors (Pereira et al. 2001). However, the numbers of animals in the group treated with *N*-methyl-*N*-nitrosourea, dichloroacetic acid, and chloroform and in the group treated with *N*-methyl-*N*-nitrosourea, trichloroacetic acid, and chloroform were variable and small ($n = 6-8$ in the female group), limiting the power of the study. In female mice, coexposure to 800 and 1,600 mg/L decreased the number of adenomas induced by *N*-methyl-*N*-nitrosourea and dichloroacetic acid, with no effect on carcinomas or on adenomas and carcinomas in the liver induced by *N*-methyl-*N*-nitrosourea and trichloroacetic acid. *N*-methyl-*N*-nitrosourea and dichloroacetic acid treatment resulted in no carcinoma induction in females. Only one animal had carcinomas induced by *N*-methyl-*N*-nitrosourea, dichloroacetic acid, and chloroform treatment. In male mice, *N*-methyl-*N*-nitrosourea and dichloroacetic acid treatment induced carcinomas as well as adenomas in the liver, with chloroform coexposure (at high concentrations) having no effect on the numbers of animals with adenomas and a reduction in those with carcinomas. Only the highest concentration of chloroform appeared to decrease the number of animals with adenomas in the trichloroacetic acid-treated group. No foci of altered hepatocytes were found in *N*-methyl-*N*-nitrosourea-initiated control mice of either sex. A larger number of foci of altered hepatocytes were seen in female than in male mice after *N*-methyl-*N*-nitrosourea and dichloroacetic acid treatment, although the number of tumors per mouse was about the same. Chloroform decreased the number of foci of altered hepatocytes and tumors per mouse at the two highest doses of dichloroacetic acid treatment in females and at the highest doses in males. Trichloroacetic acid induced few foci in female or male mice, with chloroform having no effect on foci of altered hepatocyte formation or tumor induction. Liver tumors and foci of altered hepatocytes were characterized as basophilic or eosinophilic. In females, both foci of altered hepatocytes and tumors were eosinophilic after *N*-methyl-*N*-nitrosourea and dichloroacetic acid treatment, whereas in males only foci of altered hepatocytes were eosinophilic, with tumors being basophilic. Coexposure to chloroform increased the percentage of foci of altered hepatocytes in males that were basophilic. Liver foci of altered hepatocytes and tumors induced by *N*-methyl-

N-nitrosourea and trichloroacetic acid treatment were basophilic in both sexes, with methyl chloroform having no effect. These results are consistent with those of Latendresse and Pereira (1997), who reported that, after initiation of *N*-methyl-*N*-nitrosourea, dichloroacetic acid-induced foci of altered hepatocytes and tumors in female mice were eosinophilic and stained positively for transforming growth factor alpha, *c-jun*, *c-myc*, CYP2E1, CYP4A1, and glutathione *S*-transferase (GST)-pi, while trichloroacetic acid treatment induced foci of altered hepatocytes and tumors that were predominantly basophilic, lacked GST-pi, and stained variably for other biomarkers.

Pereira et al. (2001) also reported promotion of kidney tumors in male mice from dichloroacetic acid, trichloroacetic acid, and chloroform coexposures. The pattern of tumors in the kidneys were different than in the liver. No kidney tumors were initiated in male mice after treatment with *N*-methyl-*N*-nitrosourea alone or with chloroform coexposure. However, trichloroacetic acid increased the incidence (90%) and multiplicity of kidney tumors initiated by *N*-methyl-*N*-nitrosourea. Coexposure of chloroform with trichloroacetic acid had no effect on tumor incidence or multiplicity. Dichloroacetic acid alone did not significantly increase the incidence (24%) or multiplicity of *N*-methyl-*N*-nitrosourea-initiated kidney tumors, but coexposure with chloroform increased the incidence of kidney tumors to 100% in male mice. In female mice, kidney tumor incidence and multiplicity after trichloroacetic acid or dichloroacetic acid treatment with or without chloroform was low after initiation with *N*-methyl-*N*-nitrosourea.

Using a single-dose exposure, the toxicity of a quaternary mixture of trichloroethylene, allyl alcohol, chloroform, and thioacetamide, structurally dissimilar toxicants with dissimilar mechanisms by which they initiate liver injury, was tested and compared with the toxicity of individual components and the sum of their toxic effects in male Wistar rats (Soni et al. 1999). Also, the liver reparative responses to injury initiated by each component, and the sum of their effects, were compared with the response after exposure to the quaternary mixture. The combined toxic effects were additive, primarily because of a dose-related stimulation of liver reparative response that prevented progression and expansion of liver injury. The studies showed that the extent of injury at early time points correlates well with maximal stimulation of the liver tissue repair response suggesting that, in addition to initiation of tissue injury, the toxicodynamics of cell birth and tissue repair should be considered in evaluating the final toxic outcome.

Trichloroethylene and Tetrachloroethylene

Trichloroethylene and tetrachloroethylene are often found together as environmental contaminants, are metabolized by the same enzymes, and have similar metabolites (Green 1990). There are significant differences in the kinetics of metabolism of trichloroethylene and tetrachloroethylene by certain enzymes and in the chemical reactivity of certain analogous metabolites (IARC 1995a). Tetrachloroethylene metabolites are also formed in oxidative metabolism of trichloroethylene. But trichloroethanol and chloral are less important metabolites in tetrachloroethylene than in trichloroethylene metabolism. Tetrachloroethylene appears to be a much poorer substrate for cytochrome P-450 than its congener trichloroethylene (Ohtsuki et al. 1983; Volkel and Dekant 1998; Volkel et al. 1998). Hence, the various cytochrome P-450-derived metabolites from tetrachloroethylene and trichloroethylene will be produced at different rates. In vivo, tetrachloroethylene is conjugated with reduced glutathione (GSH) more

extensively (1% to 2% of the dose) (Dekant et al. 1986a) than trichloroethylene (<0.05% of the dose) (Green et al. 1997a). In humans, the GSH conjugation pathway is toxicologically significant only at high doses or when the cytochrome P-450 pathway is saturated with trichloroethylene and tetrachloroethylene (Green 1990; Green et al. 1990). The glutathione pathway plays a greater role in tetrachloroethylene metabolism than in trichloroethylene metabolism. Chloral hydrate, a metabolite of both tetrachloroethylene and trichloroethylene, produces liver tumors in B6C3F₁ mice (Rijhsinghani et al. 1986). Although chloral hydrate is the predominant intermediate in cytochrome P-450 metabolism of trichloroethylene, it is a minor intermediate in tetrachloroethylene metabolism (Lash and Parker 2001). Such differences in rates of metabolism of trichloroethylene and tetrachloroethylene and in mode of action imply that their risk hazards differ even though the same metabolites occur with both compounds. A nongenotoxic mode of action plays an important role in liver tumorigenesis induced by trichloroethylene and tetrachloroethylene in B6C3F₁ mice. Tetrachloroethylene showed a higher degree of cytotoxicity than trichloroethylene in kidney cells isolated from male rats (Lash and Parker 2001). Low doses of trichloroethylene (5-20 μ L) or tetrachloroethylene (1-5 μ L) significantly enhanced the intracellular GSH concentration. However, the concentration of GSH rapidly decreased with higher doses of trichloroethylene (40-80 μ L) or tetrachloroethylene (10-20 μ L) (Wang et al. 2001).

Trichloroethylene and Ethanol

Because trichloroethylene and ethanol have common metabolic pathways and the liver is the main site of metabolism for both compounds, there is special interest in understanding whether individuals exposed to trichloroethylene who also consume alcohol regularly are at greater risk for developing target organ toxicity and cancer. Lower tolerance to the inebriating effects of alcohol among workers exposed to trichloroethylene has been well-documented. A condition known as “degreasers flush” is seen in subjects exposed to trichloroethylene, where dilation of blood vessels in the skin surface occurs with consumption of small amounts of alcohol (Stewart et al. 1974).

The interaction between alcohol and trichloroethylene is very complex. The outcome of this interaction depends on whether there is simultaneous or alternate exposure to the two compounds. This interaction could involve: (1) direct competition between ethanol, trichloroethylene, and its metabolites for drug-metabolizing enzymes; (2) increased expression and activity of liver CYP2E1 by alcohol consumption, which is known to affect trichloroethylene metabolism; (3) changes in availability of cofactors for enzymes catalyzing the reductive and oxidative metabolism of trichloroethylene that occurs as a result of oxidative metabolism of ethanol; and (4) abnormal generation of reactive oxygen species by induced CYP2E1, which could synergize the adverse effects of trichloroethylene metabolites.

Trichloroethylene undergoes oxidation to chloral hydrate by the action of CYP2E1. Chloral hydrate then undergoes conversion to trichloroacetic acid. This is an oxidative reaction catalyzed by aldehyde dehydrogenase, which requires oxidized nicotinamide adenine dinucleotide (NAD) as cofactor. Alternatively, chloral hydrate can be converted to trichloroethanol by alcohol dehydrogenase. This reductive reaction requires reduced nicotinamide adenine dinucleotide (NADH). Ethanol uses the same two pathways for its consecutive oxidation to acetaldehyde and acetic acid, respectively.

Oxidation by CYP450 Versus GSH Conjugation Via GST: Changes in the Contribution of These Pathways to Trichloroethylene Metabolism by Ethanol

Microsomal metabolism of ethanol via CYP2E1 occurs more prominently relative to alcohol dehydrogenase with chronic alcohol use (Lieber 2004). Competition for CYP2E1 during coexposure to ethanol and trichloroethylene can reduce the conversion of trichloroethylene to chloral hydrate (Muller et al. 1975). By blocking CYP2E1, less conversion of trichloroethylene to chloral hydrate can increase the narcotic and solvent effects of trichloroethylene in various tissues. This interference with CYP2E1 can also shift the metabolism of trichloroethylene into the glutathione pathway (Sato and Nakajima 1985), resulting in generation of more glutathione-derived adducts of trichloroethylene. Generation of more of these conjugates can alter the susceptibility of exposed subjects to the adverse effects of trichloroethylene in kidneys because greater generation and delivery of *S*-1,2-dichlorovinyl-L-cysteine to this organ can be detrimental. This metabolite has been linked to both acute tubular necrosis (Gandolfi et al. 1981; Vaidya et al. 2003a) and cancer formation by trichloroethylene. These findings are based primarily on animal studies. Additional experimentation is needed to determine whether this shift in trichloroethylene metabolism occurs with ethanol coexposure and what its toxicologic significance is in humans.

Glutathione-mediated metabolism of trichloroethylene in humans is considered a minor pathway compared with its oxidative metabolism. This is in contrast to rodents, which are considered more susceptible to the acute nephrotoxicity and nephrocarcinogenicity of trichloroethylene. Accordingly, conjugation of trichloroethylene with glutathione is more prominent in rodents than in humans (Green et al. 1997a; Lash et al. 2000a). Bernauer et al. (1996) analyzed the urine of rats and humans for the presence of the *N*-acetylated metabolite of *S*-1,2-dichlorovinyl-L-cysteine after trichloroethylene exposure via inhalation. Urinary excretion of this metabolite was compared with that of products of the oxidative metabolites of trichloroethylene. The results showed that the urinary content of *N*-acetylated *S*-1,2-dichlorovinyl-L-cysteine in humans was 1,000-7,000 times lower than that for trichloroethanol and trichloroacetic acid (Bernauer et al. 1996).

The existing data suggest that GSH conjugation is a minor pathway for the metabolism of trichloroethylene in humans, but there is no indication of whether this pathway becomes more prominent during coexposure to ethanol and trichloroethylene, when ethanol metabolism impairs the oxidative metabolism of trichloroethylene via CYP2E1. By contrast, exposure to trichloroethylene alone in alcohol users is expected to have contrasting effects on the ability of the liver to metabolize trichloroethylene. With CYP2E1 induction, chloral hydrate formation during abstinence from alcohol consumption is expected to be higher, which should lead to enhanced generation of oxidative and conjugative trichloroethylene metabolites. Several animal studies have shown that to be the case. On the other hand, human studies documenting this finding are scarce.

Shift in Reducing Equivalents During Alcohol Metabolism

Ethanol metabolism is also known to shift the balance of reducing equivalents in hepatocytes (Kalant et al. 1970). A shift in the ratio of NAD^+ to NADH in favor of the reduced pyridine nucleotide takes place during alcohol metabolism. This higher reducing environment in

hepatocytes is known to affect the oxidative metabolism of trichloroethylene, as illustrated in studies by Larson and Bull (1989) where coadministration of ethanol and trichloroethylene to male Sprague-Dawley rats resulted in decreased blood concentrations of trichloroacetic acid compared with animals receiving trichloroethylene alone. Generation of trichloroacetic acid depends on NAD^+ availability, which is lower during alcohol metabolism. Ethanol coexposure also increases the urinary excretion ratio for trichloroethanol/trichloroacetic acid (Larson and Bull 1989). The authors of the study pointed out that this effect was pronounced only when very high doses of trichloroethylene and ethanol were used. Nevertheless, the study shows that a larger supply of reducing equivalents by alcohol metabolism favors the formation of trichloroethanol over trichloroacetic acid, which was highly predictable based on the form of NADH needed to catalyze the different chloral hydrate biotransformation reactions. The effect of ethanol on trichloroethylene metabolism has also been investigated in isolated perfused rat livers (Watanabe et al. 1998). The results of these liver perfusion studies are similar to those reported by Larson and Bull (1989).

The change in the NAD^+ -to-NADH ratio produced by ethanol oxidation has another implication for exposure to mixtures beyond the changes in activity of metabolic pathways for trichloroethylene just described. This shift in reducing equivalents resulting from NADH accumulation also increases mitochondrial superoxide production by accelerating the flow of electrons down the respiratory electron transport chain (Koch et al. 2004). This, along with a higher production of reactive oxygen species under conditions of CYP2E1 induction, can enhance the susceptibility of the liver and other target organs to lipid peroxidation and oxidative damage to DNA produced by trichloroethylene and its metabolites. This shift in reducing equivalents produced by alcohol metabolism and NADH accumulation has also been implicated in some pathologic findings of alcoholic liver disease, including inhibition of fatty acid oxidation and steatosis.

CYP2E1 Induction and Oxidative Stress

The effect of alcohol use on microsomal metabolism and CYP2E1 expression deserves more in depth attention. The biochemical and toxicologic features of CYP2E1, as they relate to alcohol metabolism and toxicity, were recently reviewed by Caro and Cederbaum (2004). A decade ago, Cederbaum's group developed a human hepatoma HepG2 cell line with constitutive expression of CYP2E1. The parental cell line lacks any detectable CYP2E1. Overexpression of CYP2E1 in HepG2 cells results in a 50% increase in production of reactive oxygen species compared with untransfected cells. Associated with this, CYP2E1-expressing cells also exhibited increased lipid peroxidation and a significant decrease in cell proliferation that is possibly due to mitochondrial damage inflicted by CYP2E1-induced oxidative stress. It is worth noting that the enhanced oxidative stress in transfected cells occurs in the absence of added toxicant, which indicates that CYP2E1 expression by itself is responsible for this effect.

Although ethanol oxidation by liver alcohol dehydrogenase is the rate-limiting step in the total oxidation of this alcohol, ethanol oxidation also occurs via CYP450. This alternative metabolic pathway for ethanol is more pronounced with chronic alcohol consumption due to the well-documented CYP2E1 induction that occurs with chronic exposure. Multiple reviews have described this phenomenon and the mechanism involved in CYP2E1 induction (Lieber 2004). Normal CYP2E1 enzymatic activity generates reactive oxygen species such as superoxide and

hydrogen peroxide in higher amounts than other CYP450 isoforms (Gorsky et al. 1984). With ethanol induction of hepatic CYP2E1, the enhanced formation of reactive oxygen species resulting from normal CYP2E1 catalysis has been linked to development of chronic alcoholic liver disease. Most importantly, in vivo and in vitro studies with freshly isolated hepatocytes have also demonstrated that ethanol exposure can produce oxidative stress and hepatocellular injury.

CYP2E1 induction by ethanol has dual implications to toxicity resulting from exposure to mixtures consisting of ethanol and other xenobiotics. Enhanced expression of CYP2E1 influences not only the toxicologic potency of xenobiotics by altering the profile of metabolites that are generated but also the formation of reactive oxygen species that occurs with CYP2E1 induction can potentiate the toxic effects of xenobiotics that work by generating reactive oxygen species. These considerations are highly relevant to trichloroethylene because alcohol consumption has been reported to affect trichloroethylene metabolism and also its hepatotoxicity (Nakajima et al. 1988; Okino et al. 1991). In summary, two sources of potentially damaging reactive oxygen species have been presented in relation to alcohol consumption: (1) one coming from NADH accumulation, which stimulates mitochondrial superoxide generation, and (2) a second one originating from induced CYP2E1 enzymatic activity. Reactive nitrogen species is another category of damaging intermediates produced in response to alcohol consumption (see below).

Ethanol and Nitric Oxide Production: Changes in Blood Flow and Peroxynitrite Formation

The enhanced production of nitric oxide that occurs in association with alcohol consumption can also affect the toxicity of trichloroethylene and its metabolites. Ethanol increases blood flow to selected organs, such as the kidney and liver, without affecting perfusion to other tissues like the brain and lungs. This effect appears to be mediated by a stimulation of nitric oxide production (Baraona et al. 2002a). However, there are conflicting reports on the effect of ethanol on the activity of inducible nitric oxide synthase. Some investigations indicate that ethanol induces nitric oxide synthase activity (Baraona et al. 2002a,b), but a recent study in rats showed that consuming a liquid diet containing 3% ethanol (vol/vol) for 12 weeks reduced hepatic inducible nitric oxide synthase activity significantly (Wang and Abdel-Rahman 2005). These results are inconsistent with higher nitric oxide generation. Regardless of the mechanism involved, increased production of nitric oxide by ethanol has dual implications for the toxicity of other xenobiotics. Changes in blood perfusion rates to selected organs can lead to changes in pharmacokinetic and pharmacodynamic parameters for xenobiotics in alcohol users. Second, increased production of nitric oxide leads to secondary production of peroxynitrite. This reactive nitrogen intermediate has been shown to cause protein nitration and tissue injury (Jaeschke et al. 2002). The combined effect of peroxynitrite and reactive oxygen species generated in response to alcohol consumption can synergize the cytotoxic potential of trichloroethylene.

Implications

Ethanol coexposure can change the biotransformation and disposition of trichloroethylene through three distinct mechanisms: (1) by direct competition between chloral

hydrate and ethanol or its oxidative product acetaldehyde for alcohol or aldehyde dehydrogenase, (2) by changing the ratio of pyridine dinucleotide cofactors needed to convert chloral hydrate to trichloroacetic acid or trichloroethanol, and (3) by direct competition between trichloroethylene and ethanol for the active site of CYP2E1.

Greater availability of NADH favors the conversion of chloral hydrate to trichloroethanol, which is considered to be a noncarcinogenic metabolite of trichloroethylene. The significance to human health of this shift in metabolism is not known because most reports documenting this effect were generated with rodents. Simultaneous metabolism of trichloroethylene and ethanol by CYP2E1 can shift more of the trichloroethylene metabolism into the GST-conjugation pathway. In turn, higher generation of GSH-derived adducts of trichloroethylene (including *S*-1,2-dichlorovinyl-L-cysteine) can alter the susceptibility of the kidneys to acute necrosis and cancer. Once again, the significance of this interaction in human health is unknown.

On the other hand, exposure to trichloroethylene after alcohol consumption in habitual drinkers represents another chemical interaction with mechanistic features that are distinct from the coexposure situation. As a result of CYP2E1 induction in alcohol users, trichloroethylene metabolism to chloral hydrate proceeds faster when ethanol is not present. This has been documented in rat studies in which pretreatment with ethanol resulted in increased urinary excretion of CYP450-derived metabolites of trichloroethylene, trichloroacetic acid, and trichloroethanol (Nakajima et al. 1988). This was associated with more pronounced hepatotoxicity.

There is a large volume of data documenting this interaction between ethanol and trichloroethylene, where both metabolism and pattern of toxicity by trichloroethylene are changed. However, the bulk of this information is limited to studies using laboratory animals. Although some of the human data suggest that this interaction can occur in the workplace, its significance to alterations in patterns of trichloroethylene toxicity and cancer is unknown and deserves further attention.

POTENTIAL MECHANISMS OF INTERACTION

Bartonicek and Teisinger (1962) showed that disulfiram markedly inhibits the terminal enzymatic steps (detoxification) of trichloroethylene metabolism, resulting in enhanced trichloroethylene toxicity. Trichloroethylene induces CYP2E1 and inhibits alcohol dehydrogenase (Wang et al. 1999). Chloroform, when coadministered with dichloroacetic acid and trichloroacetic acid (metabolites of trichloroethylene), promoted kidney tumors in male mice by preventing hypomethylation of DNA and increasing mRNA expression of the *c-myc* gene (Pereira et al. 2001). The inductive and inhibitory effects of trichloroethylene on CYP2E1 and alcohol dehydrogenase, respectively, might result in different effects on the metabolism of other chemicals when coadministered with trichloroethylene. Besides a toxic response, tissue repair, a simultaneous biological compensatory response that accompanies chemical-induced injury, also plays an important role in mixture toxicity (Anand et al. 2005a,b). Trichloroethylene potentiates the hepatotoxicity of carbon tetrachloride by increasing carbon tetrachloride-induced lipid peroxidation (Pessayre et al. 1982).

Recent studies (Vaidya et al. 2003b,c; Korrapati et al. 2005) suggest another potential mechanism of altered toxicity upon coexposure to other toxicants. Renal tissue repair was

inhibited by a high dose of *S*-(1,2-dichlorovinyl)-L-cysteine (75 mg/kg, intraperitoneally) due to down-regulation of the IL-6/STAT-3 or the IL-6/ERK1/2 pathways causing cell cycle arrest at the beginning of the G₁- to S-phase transition (Vaidya et al. 2003c). Downstream of the ERK1/2 pathway, a high dose of *S*-(1,2-dichlorovinyl)-L-cysteine inhibits phosphorylation of IκBα, resulting in limited nuclear translocation of NF-κB. A cdk4/cdk6 system-mediated phosphorylation of retinoblastoma protein was down-regulated due to overexpression of p16 (Korrapati et al. 2005). Prior administration of a low priming dose of *S*-(1,2-dichlorovinyl)-L-cysteine (15 mg/kg) protects mice from a later lethal dose of *S*-(1,2-dichlorovinyl)-L-cysteine (75 mg/kg) (Vaidya et al. 2003b). A low dose of *S*-(1,2-dichlorovinyl)-L-cysteine exhibits prompt renal tubular regeneration by timely and adequate stimulation of IL-6, TGF-α, HB-EGF, EGFr, IGF-1Rβ, and phosphorylated ERK1/2, leading to recovery from a lethal dose challenge (Vaidya et al. 2003c). A priming dose led to higher expression of cyclin D1/cdk4-cdk6 downstream, resulting in increased phosphorylation of retinoblastoma protein (Korrapati et al. 2005). Coexposure to other toxicants may result in interactions with the cellular signaling mechanisms affecting the response to trichloroethylene and, conversely, trichloroethylene (or its metabolites) might interfere with the cellular signaling mechanisms and cellular responses. Effects of chronic exposure to trichloroethylene or its metabolites on cellular signaling mechanisms and how they might be altered upon coexposure to other toxicants are not known.

EFFECTS OF ALTERED OR SPECIAL PHYSIOLOGIC STATES

Studies of exposure to trichloroethylene suggest a concern about reproductive issues and congenital heart defects (see Chapter 5 for complete discussion). For mixtures containing trichloroethylene, an increase in miscarriages has been reported among nurses exposed to unspecified concentrations of trichloroethylene and other chemicals in operating rooms (Corbett et al. 1974). Early exposure of male rabbits to a mixture of arsenic, chromium, lead, benzene, chloroform, phenol, and trichloroethylene in drinking water caused acrosomal dysgenesis, nuclear malformations, lower testosterone secretion, subnormal mating desire and ability, lower sperm quality, and decreased Leydig cell function (Veeramachaneni et al. 2001). Simultaneous oral administration of trichloroethylene (0.5 mL/kg) resulted in a marked potentiation of liver injury caused by an oral dose of chloroform (0.05 mL/kg) due to delayed hepatic regeneration (Steup et al. 1993). Pretreatment with drinking water solutions containing trichloroethylene or chloroform enhances the hepatotoxicity of carbon tetrachloride in Fischer 344 rats (Steup et al. 1991). Inhalation of small concentrations of petroleum and trichloroethylene caused degenerative changes in the hepatic parenchyma cells in pregnant female Wistar rats (Duricic and Duricic 1991).

COEXPOSURE PREDICTIONS USING PBPK MODELS

An important issue is whether and the degree to which modulation of toxicity by coexposures can be quantified. PBPK models have been developed to predict possible synergy, antagonism, and additivity of effects on pharmacokinetics. Given that trichloroethylene, tetrachloroethylene, and methyl chloroform are often found together in contaminated groundwater, Dobrev et al. (2001) attempted to investigate the pharmacokinetic interactions

among the three solvents to calculate defined “interaction thresholds” for effects on metabolism and expected toxicity. Their null hypothesis was defined as competitive metabolic inhibition being the predominant result for trichloroethylene given in combination with other solvents. They used gas uptake inhalation studies to test different inhibition mechanisms. A PBPK model was developed with the gas uptake data to test multiple mechanisms of inhibitory interactions (competitive, noncompetitive, or uncompetitive) with the authors reporting competitive inhibition of trichloroethylene metabolism by methyl chloroform and tetrachloroethylene in simulations of pharmacokinetics in rats. Occupational exposures to chemical mixtures of the three solvents within their threshold limit value or time-weighted average limits were predicted to result in a significant increase (22%) in trichloroethylene blood concentrations compared with single exposures.

Dobrev et al. (2002) extended this work to humans by developing an interactive human PBPK model to explore the general pharmacokinetic profile of two common biomarkers of exposure: peak trichloroethylene blood concentrations and total trichloroethylene metabolites generated in rats and humans. Increases in the trichloroethylene blood concentrations were predicted to lead to greater availability of the parent compound for glutathione conjugation, a metabolic pathway that may be associated with kidney toxicity or carcinogenicity. A fractional change in trichloroethylene blood concentration of 15% for a combined threshold limit value for exposure to the three chemicals (25, 50, and 350 ppm of tetrachloroethylene, trichloroethylene, and methyl chloroform, respectively) resulted in a 27% increase in *S*-(1, 2-dichlorovinyl)-L-cysteine metabolites, indicating a nonlinear risk increase due to combined exposures to trichloroethylene. Binary combinations of the solvents produced glutathione-mediated metabolite amounts almost twice as high as the expected rates of increase in the parent compound blood concentrations. The authors suggested that using parent blood concentrations (a less sensitive biomarker) would result in two to three times higher (less conservative) estimates of potentially safe exposures. For detecting metabolic inhibition from tetrachloroethylene and methyl chloroform, the simulations showed trichloroethylene blood concentrations to be the more sensitive dose metric in rats, but the total of trichloroethylene metabolites was a more sensitive dose measure in humans. Finally, interaction thresholds were predicted to occur at lower concentrations in humans than in rats.

Thrall and Poet (2000) investigated the pharmacokinetic impact of low-dose coexposures to toluene and trichloroethylene in male F344 rats in vivo using a real-time breath analysis system coupled with PBPK modeling. The authors reported that, using the binary mixture to compare the measured exhaled breath concentrations from high- and low-dose exposures with the predicted concentrations under various metabolic interaction simulations (competitive, noncompetitive, or uncompetitive inhibition), the optimized competitive metabolic interaction description yielded an interaction parameter K_i value closest to the Michaelis-Menten affinity parameter (K_m) of the inhibitor solvent. They suggested that competitive inhibition is the most plausible type of metabolic interaction between these two solvents.

Isaacs et al. (2004) reported gas uptake coexposure data for chloroform and trichloroethylene. They questioned whether it was possible to use inhalation data in combination with PBPK modeling to distinguish between different metabolic interactions using sensitivity analysis theory. They reported that chloroform and trichloroethylene act as competitive inhibitors of each other's metabolism. Recommendations were made for the design of efficient experiments aimed at determining the type of inhibition mechanisms resulting from a binary coexposure protocol. Even though, as stated by Dobrev et al. (2002), other solvents inhibit

trichloroethylene metabolism, it is possible to quantify the synergistic interaction of trichloroethylene on other solvents with techniques such as gas uptake inhalation exposures.

Haddad et al. (2000) developed a theoretical approach to predict the maximum impact that a mixture consisting of coexposure to dichloromethane; benzene; trichloroethylene; toluene; tetrachloroethylene; ethylbenzene; *m*-, *p*-, and *o*-xylene; and styrene would have on venous blood concentration due to metabolic interactions in Sprague-Dawley rats. They conducted two sets of experimental coexposures. The first study evaluated the change in venous blood concentration after a 4-hour constant inhalation exposure to the 10-chemical mixture. The second study was designed to examine the impact of possible enzyme induction by using the same inhalation coexposure after a 3-day pretreatment with the same 10-chemical mixture. The resulting venous concentration measurements for trichloroethylene from the first study were consistent with metabolic inhibition. The 10-chemical mixture was the most complex coexposure used in this study. The authors stated that resulting parent concentration time courses change less as mixture complexity increases, an observation consistent with metabolic inhibition. For the pretreatment study, the authors found a systematic decrease in venous concentration (due to higher metabolic clearance) for all chemicals except tetrachloroethylene. Overall, these studies suggest a complex metabolic interaction between trichloroethylene and other solvents.

A PBPK model for trichloroethylene including all its metabolites and their interactions can be considered a mixture model in which all metabolites have a common starting point in the liver. An integrated approach is needed after taking into account trichloroethylene metabolites and their interactions with each other, including inhibition of metabolites.

FINDINGS AND RECOMMENDATIONS

Although the available data indicate that toxic effects of trichloroethylene and its metabolites are likely to change in the presence of exposure to other chemicals, including its metabolites and similar metabolites of other toxicants, a definitive understanding of whether and which of the toxic effects might be increased, decreased, or unchanged is lacking. Much of this must come from research in animals or other biosystems, because in humans, exposures to other compounds and factors would be difficult to obtain accurately and reliably in humans. The present state of knowledge does allow identifying the major potential mechanisms as the basis of such interactions at the biophase, but to what extent and how they could influence the toxicity outcomes cannot be predicted. Examples of such mechanisms are altered xenobiotic metabolizing enzymes, toxicokinetic factors (absorption, distribution, and elimination), toxic metabolite accumulation in target and nontarget tissues, and toxicodynamic factors, such as cell death, proliferation, expression of survival factors, and epigenetic and genotoxic mechanisms.

Recommendations: Toxicokinetic and toxicodynamic studies are needed with mixtures to evaluate the effect of coexposures to other chemicals on toxic outcomes of trichloroethylene and on the toxicity of other coexposed toxicants including metabolites of trichloroethylene.

- Important toxic outcomes of trichloroethylene might be selected as end points for these studies. Species differences should be investigated.
- Testing large numbers and doses of compounds is not practical. Studies designed to learn more about mechanisms and modes of action in the presence of the most commonly occurring toxicants are likely to yield the most meaningful results.

- Testing to evaluate the impact of lifestyle factors, such as alcohol consumption, smoking, chronic drug intake, and diet (e.g., nutrition, caloric restriction) should be performed.
- Testing of mixtures to evaluate the impact of disease (e.g., diabetes) and special physiologic states (e.g., pregnancy, aging) should be performed.

11

Pharmacokinetic Modeling

Pharmacokinetic models describe the absorption, distribution, metabolism, and elimination of a chemical in an organism. Depending on the complexity of a pharmacokinetic model and the available data upon which it is based, the model can be used to predict the concentration of a parent chemical and metabolite(s) in various tissues, organs, cells, and subcellular compartments given any particular exposure pattern over time. Because target organ doses are more relevant to toxicity than the amount of exposure at a particular exterior boundary, pharmacokinetic models may be useful for assessing human health risk from exposure to a chemical or mixture of chemicals with shared metabolic pathways.

In keeping with the committee charge, this chapter discusses key scientific issues regarding approaches for pharmacokinetic modeling of trichloroethylene based on existing metabolic information and uses of pharmacokinetic modeling results for trichloroethylene risk assessment. Discussion of approaches to pharmacokinetic modeling of trichloroethylene focuses on (1) the relative strengths and weaknesses of different model structures and parameterization, including the tradeoff between model complexity (and hence completeness) and uncertainty, and (2) the evaluation of model uncertainties. Discussion of the uses of pharmacokinetic modeling results for risk assessment of trichloroethylene focuses on (1) dose metrics for developing human equivalent doses, route-to-route extrapolation, and use in biologically based dose-response modeling; and (2) uncertainties associated with pharmacokinetic-based dose metrics and consideration of non-pharmacokinetic-based scaling approaches.

This chapter does not include an exhaustive review of the literature on pharmacokinetic models for trichloroethylene. The pharmacokinetic models used in the U.S. Environmental Protection Agency (EPA 2001b) draft health risk assessment of trichloroethylene, several of the pharmacokinetic models published since that assessment, and a model later commissioned by EPA and the U.S. Air Force (USAF) to deal with some problems of the earlier health risk assessment are the focus of this chapter.

OVERVIEW OF PHARMACOKINETIC MODELS

Pharmacokinetic models mathematically describe the absorption, distribution, metabolism, and elimination of a chemical in an organism as a function of time. Similar descriptions for metabolites also can be incorporated into pharmacokinetic models for the parent compound. Pharmacokinetic models typically include compartments that represent specific organs and tissues as well as lumped tissue compartments and are represented by using systems of differential equations. Whether a specific tissue compartment is included in a pharmacokinetic model depends on how involved that tissue is in disposing of the compound (e.g., portals of entry or excretion, sites of metabolism, targets of toxicity) and on its utility as a biomarker of exposure or response. Pharmacokinetic model development is an iterative process; the mathematical model is used to simulate data and the simulated data are compared with real data to refine the mathematical model. “All models are wrong, but some models are useful” (attributed to G. Box [Kokko 2005]). There will never be a comprehensive model that perfectly describes all the exposure and response relationships for any chemical in laboratory animals or humans, but some models may be adequate for predicting useful internal dose metrics, and some models may provide better predictions than others.

Physiologically based pharmacokinetic (PBPK) models define model parameters in terms of directly interpretable anatomic, physiologic, or biochemical quantities. In a basic PBPK model, the tissue compartments are linked by blood flow and have associated physical volumes and partition coefficients that describe the relative degree to which a given chemical (e.g., trichloroethylene) is soluble in each of those tissues versus blood. Although the fundamental mathematical forms of pharmacokinetic and PBPK models with the same compartments may be identical, parameterization in terms of measurable physiologic quantities introduces several advantages (Gibaldi and Perrier 1982). For example, blood flow rates are well characterized in many species, providing a simple and rational method for adjusting a PBPK model to extrapolate across species (e.g., from laboratory animals to humans). Moreover, direct measurements can be independently obtained for some PBPK parameters, rather than relying solely on the results of dosing experiments.

The complexity of a pharmacokinetic model depends on the availability of data, the certainty and confidence in the scientific understanding of the processes described by the model, and the intended use of the model. In general, one begins with the simplest model that describes the data and adds complexity to the structure based on experimental data, lack of model fit to the data, and lack of model applicability to a specific end point of interest. For example, if one is interested only in evaluating the concentration of the parent compound in blood and other tissues over time, the model structure can be very simple. Disappearance of the parent chemical may be described by a “whole-body” metabolism rate and details on different metabolic pathways are not necessary. Pharmacokinetic models are powerful tools that can be used to identify data gaps and research needs. As mechanistic hypotheses are developed, modifications to the model may be necessary to describe a more appropriate dose metric. For example, if one is interested in looking at the effects of a putative toxic metabolite on a specific organ (e.g., kidney), the model structure will likely be more complex.

To account for total body mass (and volume) and total cardiac output (flow), pharmacokinetic models typically include “lumped” tissue compartments that are not relevant to describe a particular chemical. For example, brain, muscle, and skin are usually not included as discrete compartments unless those tissue concentrations are direct targets for prediction (e.g.,

brain concentration for predicting neurotoxicity). Instead, tissues are lumped as richly (or rapidly) and poorly (or slowly) perfused tissue groups. The richly perfused tissue group typically includes organs and tissues such as the brain, kidney, and alveolar region of the lungs, and the poorly perfused group includes tissues such as muscle and skin.

Advantages and Limitations

PBPK models hold particular promise in assessing human health risks from multiple routes of exposure to the same chemical or from exposures to related chemical mixtures, because of their ability to predict doses at the tissues where relevant toxic effects occur. Traditional metrics such as the lifetime average daily dose fail to reflect differences in metabolism and disposition by exposure route, particularly when metabolic rates are saturable or when multiple exposures occur simultaneously. Theoretically, an accurate PBPK model is ideal for characterizing human health risk from a complex exposure pattern involving chemical mixtures, multiple exposure routes, saturable binding and metabolism, and any other situation resulting in a nonlinear relationship between the exposure metric and the target organ dose.

PBPK models are difficult to develop, limited to predicting concentrations in particular tissues, and imperfectly model the processes their creators seek to describe. Compartments with different characteristics often must be grouped together to make parameterization and analysis feasible. Simplifying assumptions may be made without confirmation to establish parametric differential equations (e.g., the steady-state assumption used to derive the Michaelis-Menten equation, as per Gibaldi and Perrier [1982], or the common assumption of flow-limited exchange). Imperfect models can still be quite useful (Morgan et al. 1990), but model predictions for species or exposure patterns other than those used to develop the model should be interpreted with caution after giving careful attention to model assumptions.

Pharmacokinetic model building is a difficult task, as for any complex inference problem (Neter et al. 1996). Specification of few compartments in a pharmacokinetic model facilitates direct and precise statistical estimation of parameters, provides quick results, and may be sufficient for many purposes. However, oversimplification may lead to a biased prediction. Because health risk assessment is concerned with prediction rather than hypothesis testing or other forms of inference, loss of precision in parameter estimates is acceptable to avoid bias in the prediction. Although specifying many compartments decreases the chance of biased prediction when parameters are estimated solely from dosing data, typical PBPK model parameterization often relies on external estimates in addition to direct fits to dosing data and therefore may not protect so strongly against bias through additional compartments. Bayesian statistical approaches may be particularly advantageous for complex pharmacokinetic systems, allowing one to formally incorporate external information and propagate uncertainties, while relying on experimental data to drive the final parameter estimates toward unbiased values (Wakefield and Rahman 2000).

Flow- Versus Diffusion-Limited Exchange

Flow-limited exchange describes the situation in which a chemical is assumed to always be at dynamic equilibrium between the tissues represented by a compartment and venous blood

leaving those tissues. In this situation, the exchange rate between the blood and the compartment is limited primarily by the blood flow rate to the tissues represented by that compartment. Flow-limited exchange appears to be a default assumption in many PBPK models, but it is sometimes an oversimplification (O'Flaherty 1991). In contrast, diffusion-limited exchange does not solely depend on blood flow rates and may be limited by rates of bone accretion or other processes. Tissues that exhibit diffusion-limited exchange may not be well characterized using a single compartment, in which case they are represented using many compartments connected in layers (O'Flaherty 1991), membrane diffusion models (McCarley and Bunge 2001), or other approaches. PBPK model builders and users should carefully assess default assumptions of flow-limited exchange.

Parameterization of PBPK Models

PBPK models contain two basic parameter types: physiologic and chemical specific. Physiologic parameters describe the organism and include parameters such as body weight, blood flow (total cardiac output as well as flow to different organs and tissues), tissue and organ volumes, and respiratory rates. These parameters are usually specific to a given species and are gleaned from the literature rather than measured. Examples of references from which physiologic parameters for PBPK models are obtained include Brown et al. (1997) and Arms and Travis (1988). Allometric scaling is frequently used in PBPK models for volumes and flows when scaling from animals to humans.

As the name implies, chemical-specific parameters are unique for each chemical and include physicochemical parameters (e.g., tissue partition coefficients) and biochemical parameters (e.g., metabolic rate constants, absorption and excretion rates). Tissue partition coefficients describe the extent to which a chemical is soluble in various fluids and tissues. Ideally, partition coefficients are determined experimentally for each test article and in tissues from each species to be modeled. Partition coefficients for a "typical" tissue may be used as the partition coefficient in a lumped tissue compartment (e.g., liver tissue partition coefficient may be used for the liver and the lumped "richly perfused" tissue compartment). Partition coefficients also can be estimated indirectly by using known chemical properties. For example, Poulin and Krishnan (1996) developed algorithms to deterministically estimate tissue partition coefficients based on the lipid solubility of the chemical and the fat content of the tissue. Tissue partition coefficients also may be estimated based on structurally similar chemicals (or classes of chemicals) (Beliveau et al. 2003). It is possible for partition coefficients for a given chemical to vary with species and even gender within a species. However, in the absence of data to the contrary, it is often assumed that tissue partition coefficients are no species specific (e.g., the partition coefficient in the liver of a mouse is no different from that in a human).

Biochemical parameters also are chemical specific and include parameters such as absorption and excretion rate constants and metabolic rate constants (e.g., first-order rate constant, k ; Michaelis-Menten rate constants, K_m and V_{max}). Biochemical parameters may be measured or estimated based on a fit to experimental data. Allometric scaling across species is used to estimate biochemical parameters when data are not available for the species of interest.

Uncertainty

Typical PBPK models include many unknown parameters and often highly multimodal likelihood surfaces, leading to challenging inference problems. In particular, parameter uncertainty can complicate inference. Possible strategies include deterministically fixing underdetermined parameters and restricting parameters to biologically meaningful constraints. Some of these strategies were used in a newly available model discussed below (USAF-EPA 2004a).

Alternatively, one could describe uncertainties in the form of probability distributions on unknown parameters. This leads to an approach known as Bayesian statistical inference. Bayesian statistics approaches inference for a random process by expressing uncertainty about unknown parameters as subjective probabilities. For example, the parameters could be unknown biochemical parameters.

The probability distribution describing uncertainty of the parameters before observing any data is known as the prior probability distribution. After observing data, the prior distribution is updated by using the rules of probability calculus. The updated probability distribution of the parameters is known as the posterior distribution. It contains all relevant information about the unknown parameters. From a Bayesian perspective, all statistical inference can be deduced from the posterior distribution by reporting appropriate summaries. In particular, this includes predictive inference.

In the context of parameterized physical systems, like the PBPK model, posterior inference and simulation for the unknown parameters are also described as the Monte Carlo method to solving the inverse problem (Mosegaard and Tarantola 1995). A recent discussion of this strategy appears in studies of Cornford et al. (2004), Haario et al. (2004), and Robert (2004).

Variability

Uncertainty is distinct from variability inherent to the described process. For example, PBPK models can include subject-specific parameters and describe subject-to-subject variation. This is formalized as a random effects distribution of subject-specific parameters. The variability of this distribution is inherent to the process. Even infinite data would never reduce this variability to zero.

Describing such variability takes the form of a hierarchical extension of the basic model. Let θ denote the subject-specific parameters, and let $p(y | \theta)$ denote the sampling model for the observed data, given the set of PBPK parameters θ . The model is hierarchically extended with a second layer $\theta \sim p(\theta | \mu)$ to describe intersubject variability, where μ represents a set of unknown population parameters. In the context of population pharmacokinetic models, this strategy is described, for example, by Wakefield and Rahman (2000). The general framework is also known as mixed-effects modeling.

TRICHLOROETHYLENE PHARMACOKINETIC MODELS AND RISK ASSESSMENT

A number of pharmacokinetic models for trichloroethylene have been published over the last 30 years. During that time the amount of data from humans and experimental animal models increased significantly and these data improved the scientific understanding of trichloroethylene metabolism and the mode of action of trichloroethylene toxicity, which resulted in increased complexity of the pharmacokinetic models for trichloroethylene and its metabolites.

Exposure to trichloroethylene has been associated with a wide variety of adverse health effects including liver toxicity, kidney toxicity, reproductive and developmental toxicity, neurotoxicity, and immunotoxicity as well as cancer of the liver, kidney, lung, testes, and immune system (lymphoma). Trichloroethylene metabolism is complex (Lash et al. 2000a). As discussed in other chapters, specific metabolites have been causally associated with toxic or carcinogenic responses in different tissues and in different species. There are two major pathways for trichloroethylene metabolism: the oxidative (or cytochrome P-450) pathway and the glutathione-dependent pathway (see also Chapter 1). The flux through these two pathways differs in each tissue and the data suggest that the mode of action, including the putative toxic metabolite (dichloroacetic acid, trichloroacetic acid, chloral, and dichlorovinylcysteine), varies for different end points. To further complicate the picture, trichloroethylene metabolism varies in different species and the mode of action for a given end point also may vary with species (see details in other chapters). Clearly, there is considerable uncertainty and lack of consensus in the scientific community about the mode of action for different end points.

Other factors that complicate the assessment of human health risk from exposure to trichloroethylene include coexposure to other solvents, alcohol consumption, disease states that alter trichloroethylene metabolism and toxicity, interindividual variability in trichloroethylene metabolism, and age. There are also direct and indirect exposures to the putative toxic metabolites of trichloroethylene. For example, dichloroacetic acid and trichloroacetic acid are by-products of water chlorination and are often present in drinking water at very low concentrations, some individuals are directly exposed to chloral via medicinal use, and other parent compounds produce some of the same metabolites as trichloroethylene.

As noted above, trichloroethylene and its metabolites have been associated with toxicity and carcinogenicity in one or more species. The targets of toxicity are not the same for all species and the mode of action for the various toxic end points is not well understood. A comprehensive pharmacokinetic model for use in human health risk assessment would incorporate all potential routes of exposure, target organs, and putative toxic metabolites. Such a model would be unrealistically complex and would require substantial effort to develop and validate. Ideally, the pharmacokinetic model would be linked to a biologically based pharmacodynamic model that describes the mode of action; the linked models would yield a pharmacokinetic-pharmacodynamic model. Because pharmacokinetic-pharmacodynamic models rarely describe all adverse effects, simpler models are developed and iteratively refined to improve their ability to predict human health risk.

The EPA (2001b) draft risk assessment for trichloroethylene included pharmacokinetic models published by Fisher (2000) and Clewell et al. (2000). Since the EPA draft risk assessment was published, EPA and USAF commissioned a work group to develop a “harmonized” pharmacokinetic model for trichloroethylene and its metabolites. The work group comprised scientists from EPA, the USAF, Toxicology Excellence for Risk Assessment (TERA),

and others under contract to the USAF (USAF-EPA 2004a). The work group included Drs. Clewell and Fisher. Other investigators have published pharmacokinetic models for trichloroethylene and metabolites since the 2001 EPA draft risk assessment. Several of the models are discussed below.

Review of Several Trichloroethylene Models

Fisher Models

Fisher (2000) reviewed selected pharmacokinetic models for trichloroethylene in mice and humans, focusing on liver cancer as the outcome of interest for risk assessment. As noted in Chapter 4, trichloroethylene causes liver cancer in mice but not in rats, and trichloroacetic acid is considered the principal metabolite responsible for trichloroethylene-induced liver cancer in mice. Fisher's first-generation model includes a four-compartment description of trichloroethylene disposition (liver, fat, richly perfused, and slowly perfused tissue compartments), saturable oxidative metabolism of trichloroethylene, and a simple one-compartment model for trichloroacetic acid in the liver.

Fisher's second-generation model includes six tissue compartments (a lung compartment was added as it is a target organ in mice, and a kidney compartment was added to describe urinary excretion of trichloroethylene metabolites) and a four-compartment submodel for each trichloroethylene metabolite. The second-generation model for mice included trichloroethylene, chloral hydrate, trichloroacetic acid, dichloroacetic acid, trichloroethanol, and trichloroethanol glucuronide. In the second-generation model for humans, only trichloroethylene, trichloroacetic acid, and trichloroethanol were described. As in the first-generation models, all metabolism was assumed to occur in the liver for both species in the second-generation model. Neither Fisher model described metabolism via the glutathione pathway. The putative toxic metabolite in the kidney is formed via the glutathione pathway. Because no renal toxicity has been observed in mice, this pathway is not relevant for the mouse pharmacokinetic model. However, it may be important in human kidney toxicity.

Both Fisher models include inhalation and oral exposure to trichloroethylene. Dose metrics in the first-generation model were peak concentrations (C_{\max}) and area under the curve (AUC) for trichloroethylene in whole blood and trichloroacetic acid in plasma. Dose metrics in the second-generation model included C_{\max} and AUC for trichloroethylene and trichloroacetic acid in whole blood, trichloroethylene and metabolites in tissues, and urinary excretion of trichloroethylene and metabolites.

Clewell Model

The Clewell et al. (2000) pharmacokinetic model structure for trichloroethylene in mice, rats, and humans is much more complex than the Fisher models and includes submodels for metabolites in the three principal target tissues for cancer identified in animal bioassays: lung for chloral, kidney for dichlorovinylcysteine, and liver for chloral, trichloroacetic acid, dichloroacetic acid, trichloroethanol, and trichloroethanol glucuronide. The model for trichloroethylene includes tracheobronchial, fat, rapidly perfused, slowly perfused, liver, and

gastrointestinal tract compartments. The gastrointestinal tract is composed of the gut lumen, stomach lumen, and gut tissue; this more complex description of the gastrointestinal tract allowed a better fit to experimental data on oral absorption of trichloroethylene in a corn oil vehicle. In addition, the Clewell model links metabolism in tracheobronchial tissue to lung toxicity and metabolism in the liver to liver and kidney toxicity. Like the Fisher models, the Clewell model includes inhalation and oral exposure to trichloroethylene, but the mathematical description of oral absorption is different for the Fisher and Clewell models. The common dose metrics of C_{\max} and AUC for trichloroethylene and metabolites in various tissues, as well as urinary excretion, can be calculated with Clewell's model. In addition, the Clewell model includes the ability to calculate the lifetime average daily dose for different metrics (e.g., trichloroacetic acid AUC in liver) and time above a critical concentration for a specific analyte in a specific tissue. Specific dose metrics are discussed for liver cancer (e.g., lifetime average daily dose for trichloroacetic acid AUC and dichloroacetic acid AUC in plasma as a surrogate for liver; C_{\max} for trichloroacetic acid and dichloroacetic acid in liver), kidney cancer (lifetime average daily dose for production of reactive metabolites), lung cancer (e.g., lifetime average daily dose for chloral AUC and C_{\max} for chloral in the tracheobronchial region), and non-cancer end points.

In contrast to the Fisher models, the Clewell models for rats, mice, and humans include the glutathione pathway of trichloroethylene metabolism. The Clewell model also includes descriptions for metabolites in relevant tissues. Chloral is formed from trichloroethylene and is further metabolized in the lung compartment. 1,2-Dichlorovinylcysteine is formed in the kidney, where it causes cytotoxicity or is further metabolized and excreted in urine. Trichloroethylene undergoes oxidative metabolism in the liver to form chloral, trichloroacetic acid, dichloroacetic acid, trichloroethanol, and trichloroethanol glucuronide. There is also a description of enterohepatic recirculation of trichloroethanol glucuronide:trichloroethanol.

Harmonized Model

After publication of EPA's draft health risk assessment for trichloroethylene, a joint USAF-EPA (2004a) prepared a harmonized pharmacokinetic model for trichloroethylene and its metabolites in rats, mice, and humans. Before publication, a draft of the harmonized model was reviewed by a panel of expert scientists, whose comments were considered in the final harmonized model (USAF-EPA 2004b). The harmonized model includes a primary model for the parent compound (trichloroethylene), which is very similar in structure to the Clewell model, and a number of submodels for specific tissues (e.g., tracheobronchial and liver compartments) and specific metabolites (trichloroethanol, trichloroethanol glucuronide, trichloroacetic acid, dichloroacetic acid, and 1,2-dichlorovinylcysteine). The parent trichloroethylene model includes tracheobronchial, rapidly perfused, slowly perfused, fat, gastrointestinal tract (including stomach and duodenum for description of trichloroethylene absorption administered by corn oil gavage), and liver tissue compartments. The harmonized model accommodated oral (bolus and drinking water), inhalation, and intravenous routes of trichloroethylene exposure. The model also has the capability to describe fat as a diffusion-limited tissue compartment.

Like the Clewell model, the harmonized model includes metabolism to chloral in the tracheobronchial region, hepatic metabolism of trichloroethylene to trichloroacetic acid, dichloroacetic acid, trichloroethanol, and trichloroethanol glucuronide, and enterohepatic

recirculation of trichloroethanol glucuronide:trichloroethanol. Unlike the Clewell model, the harmonized model includes hepatic metabolism of trichloroethylene to 1,2-dichlorovinylcysteine and does not include a separate kidney compartment for 1,2-dichlorovinylcysteine. Instead, it is assumed that 1,2-dichlorovinylcysteine is formed in the liver and ends up in the kidney where it can result in toxicity or be further metabolized and excreted in urine as *N*-acetyldichlorovinylcysteine.

Dose metrics in the harmonized model include the concentration of trichloroethylene in blood and tissues, trichloroethylene AUC in blood, instantaneous concentration and AUC for chloral in the tracheobronchial region (dose metric for lung), total amount of trichloroethylene metabolized normalized to body weight (dose metric for metabolism), concentrations and AUC for trichloroacetic acid in plasma and liver (dose metric for liver cancer), concentration and AUC for trichloroethanol in blood (dose metric for non-cancer end points in liver), and total production of a thioacetylating intermediate from 1,2-dichlorovinylcysteine normalized to kidney volume (dose metric for kidney cancer).

Authors of the harmonized model state that it should be useful in risk assessment for end points where the mode of action involves tissue exposure to trichloroethylene, trichloroacetic acid, and trichloroethanol; they acknowledge that other dose metrics (e.g., chloral in lung and 1,2-dichlorovinylcysteine in kidney) are highly uncertain because of a lack of adequate pharmacokinetic data.

Poet Model

None of the above models includes a description of dermal absorption of trichloroethylene. Because trichloroethylene is found in drinking water, there is dermal exposure to trichloroethylene when bathing. It has been shown for other volatile organic compounds in chlorinated drinking water (e.g., chloroform) that dermal absorption occurs in addition to absorption via the respiratory tract when showering (Jo et al. 1990). Poet et al. (2000) incorporated dermal exposure to trichloroethylene in rats and humans and their pharmacokinetic model for trichloroethylene included experimentally determined dermal permeability coefficients for both species. Because humans are exposed to trichloroethylene by the oral, inhalation, and dermal routes, dermal exposure should be included when assessing potential risk from trichloroethylene exposure. Additional data sets in experimental animals and humans after dermal exposure to trichloroethylene may be required.

Albanese Models

Albanese et al. (2002) published a series of models that included different descriptions of the adipose compartment. These models include a standard perfusion-limited compartmental model for adipose, a diffusion-limited model, and a hybrid model with an axial-dispersion model for adipose tissue. However, as noted by the expert reviewers of the harmonized model, it may not be necessary to move away from a diffusion-limited adipose tissue compartment if the model fit to experimental data is not improved (USAF-EPA 2004b). The Albanese paper shows only model simulations of trichloroethylene concentrations in adipose tissue and no comparisons with experimental data.

Simmons Model

Simmons et al. (2002) published a pharmacokinetic model for trichloroethylene in Long-Evans rats that focused on evaluating the neurotoxicity of trichloroethylene. This five-compartment pharmacokinetic model included brain, fat, slowly perfused tissue, rapidly perfused tissue, and liver. Partition coefficients for trichloroethylene in blood, fat, muscle, brain, and liver were determined for the Long-Evans rats. Male rats were exposed to trichloroethylene by inhalation, and blood and tissues were analyzed for trichloroethylene concentrations over time. Gas-uptake studies were conducted and the model was used to optimize V_{\max} based on a fit of model simulations for trichloroethylene concentrations in the chamber. The model was then used to simulate blood, liver, brain, and adipose tissue concentrations of trichloroethylene and was compared with observed concentrations of trichloroethylene in those tissues during exposure to trichloroethylene vapors (200-4,000 parts per million). As noted in Chapter 6, trichloroethylene neurotoxicity is attributed to peak trichloroethylene concentrations in brain. This model provides a reasonable fit to the experimental data. If a pharmacokinetic model is to be used to estimate neurotoxicity in humans exposed to trichloroethylene, including a brain compartment is necessary.

Keys 2003 Model

Fisher and colleagues continue to refine earlier versions of their trichloroethylene pharmacokinetic models. An expanded model was published in 2003 (Keys et al. 2003). This model included compartments for lung, heart, brain, kidney, slowly perfused tissue, fat (diffusion limited), rapidly perfused tissue, spleen, gastrointestinal tract, and liver (deep and shallow compartments). The model accommodated oral, inhalation, and intra-arterial exposure and provided for exhalation and metabolism of trichloroethylene. The pharmacokinetics of trichloroethylene in male Sprague-Dawley rats was characterized during and after inhalation exposure to trichloroethylene and after oral or intra-arterial administration of trichloroethylene. Trichloroethylene concentrations in blood and tissues were determined. Trichloroethylene metabolites were neither measured nor modeled. As noted above for the Simmons model, including a brain compartment is advisable if one is to use a pharmacokinetic model to assess neurotoxicity risk from trichloroethylene exposure.

Keys 2004 Model

Dichloroacetic acid is formed *ex vivo* from trichloroacetic acid (Merdink et al. 1998); hence, the validity of data from early studies in which dichloroacetic acid was measured in animals and people exposed to trichloroethylene has been questioned. The harmonized model has a very simple dichloroacetic acid submodel, in part due to the questioned validity of experimental data on the concentration of dichloroacetic acid in blood and tissues after trichloroethylene exposure. As noted above, there is direct exposure to dichloroacetic acid in drinking water and dichloroacetic acid pharmacokinetics have been studied (Curry et al. 1985, 1991; Gonzalez-Leon et al. 1997, 1999; Saghir and Schultz 2002; Schultz et al. 2002). A pharmacokinetic model of dichloroacetic acid was developed that includes a description of the

ability of dichloroacetic acid to inhibit its own metabolism by suicide inhibition of glutathione *S*-transferase zeta (Keys et al. 2004). Studies were done in animals exposed to dichloroacetic acid as a parent compound rather than as a metabolite of trichloroethylene, which bypasses questions related to *ex vivo* production of dichloroacetic acid from trichloroacetic acid. Addition of this dichloroacetic acid submodel to the harmonized model will be useful only if experimental data with a high degree of accuracy for blood and tissue dichloroacetic acid concentrations are available.

Dose Metrics

PBPK-based human equivalent doses offer a sensible biologically based approach to adjusting for differences across species but may not improve accuracy if an incorrect dose metric is used. For example, AUC for the target organ concentration as a function of time is a reasonable metric in theory, assuming that the effective damage to the target organ is cumulative and occurs at a rate proportional to the target organ concentration. However, other metrics can be proposed that are just as reasonable, such as the AUC for the log of the target organ concentration. If the toxicologic process leading to tissue damage occurs at a rate proportional to the log concentration, the AUC log concentration would likely be a better measure of exposure. Tissue repair or other compensating mechanisms could suggest alternative metrics, such as an AUC for target organ concentrations exceeding a certain threshold. In practice, it is difficult to know the best metric without experiments designed to compare the predictive ability of different metrics or without understanding the mechanisms of toxicity in detail.

Similarly, for toxicants such as trichloroethylene that have several potentially toxic metabolites, it is difficult to determine which metabolite(s) contributes to any particular health effect. Current dosing experiments are suggestive but were not designed to answer either of these questions. Although PBPK modeling is well motivated and is starting to fill in some gaps in animal-to-human and cross-route extrapolation, trichloroethylene dose-response models based on PBPK modeling are best viewed as plausible, rather than superior models, among many alternatives.

This note of caution is not intended to discourage the continued development and application of PBPK models for trichloroethylene. In fact, the EPA (2001b) risk assessment for trichloroethylene presents a sophisticated and appealing application of PBPK modeling and generally presents those results in an appropriate manner. Researchers should embrace the challenges posed by multiple metabolites and the complexity of the PBPK model predictions, as they suggest a variety of useful experiments with various dose patterns to produce different target organ concentration-time profiles or different ratios of metabolites. Aggressive experimentation in this direction should yield substantial information about the mechanisms of toxicity, best target organ dose metrics, and dose-response relationships for trichloroethylene. Hack et al. (in press) discuss how Bayesian posterior inference in the PBPK model identifies parameters with a high degree of uncertainty and suggest that future kinetic studies be designed to learn about these parameters.

Uncertainty

Hack et al. (in press) describe inference in the harmonized PBPK model (USAF-EPA 2004a), formalized under the Bayesian paradigm by reporting posterior inference. This is a natural and convenient choice for a large hierarchical model of this type (Gelman et al. 1995).

First, the model is extended to a population PBPK model by adding a random effects distribution $p(\theta | \mu)$ for subject-specific PBPK parameters θ . Specifically, the population PBPK model is defined by introducing normal and lognormal random effects models $p(\theta | \mu)$ for all parameters. The model is completed with conjugate hyperpriors $p(\mu)$. A distinguishing feature of the PBPK model is the physiologic interpretation of the parameters. To ensure meaningful interpretation of the implementation, Hack et al. (in press) restrict parameters to a biologically meaningful domain. This is reasonable and appropriate.

Once the model is specified, estimating the model reduces to inference about the parameters. The use of least squares point estimators is limited by the large number of parameters and small amounts of data. The use of least-squares estimation is reported after imposing constraints for several parameters (Hack et al. in press). This is reasonable for an ad hoc first estimate, but it is important to follow up with a model refinement. This is implemented by Hack et al. by reporting posterior distributions on the unknown parameters. Posterior Markov chain Monte Carlo simulation was used to implement Bayesian posterior inference—again, a natural choice and almost a compulsory consequence of the other two choices (given the difficulties of frequentist estimation in this setting).

The basic idea of Markov chain Monte Carlo simulation is the following. It can be argued that under the Bayesian paradigm most inference takes the mathematical form of expectations of some function of interest with respect to the posterior distribution. For example, a point estimate for a parameter θ is reported as the expectation of θ under the posterior probability model (that is, an integral with respect to the posterior distribution). Similarly, predictive inference can be written as an expectation of the sampling model with respect to the posterior distribution on the unknown parameters. The problem is that these integrals typically are analytically intractable. Markov chain Monte Carlo simulation instead evaluates the desired posterior integrals as sample averages. The sample average is defined as an average over iterations in a computer simulation of a Markov chain that is set up so that the desired posterior distribution is the stationary distribution. Ergodic averages with respect to the simulated Markov chain serve to estimate the posterior integrals. For example, point estimates of parameters are represented as ergodic averages of these parameters over the Markov chain simulation. An important practical advantage of the outlined strategy is the ability to implement inference in nearly any probability model and the possibility to report inference on any event of interest. Markov chain Monte Carlo simulation was introduced by Gelfand and Smith (1990) as a generic tool for posterior inference. See Gilks et al. (1996) for a review.

In the context of PBPK models, the outlined strategy can be carried out as described by Hack et al. (in press). The simulation program MCSim (Bois and Maszle 1997) was used to implement Markov chain Monte Carlo posterior simulation in the extended model. Simulation-based parameter estimation with Markov chain Monte Carlo posterior simulation gives rise to an additional source of uncertainty. Ergodic averages computed from the Markov chain Monte Carlo simulation output represent the desired posterior means only asymptotically, in the limit as the number of iterations goes to infinity. Any implementation needs to include a convergence diagnostic to judge practical convergence. Hack et al. report use of the convergence diagnostic

of Gelman et al. (1996). Although the reported diagnostic statistics are not perfect, the committee finds that they are adequate in light of the highly computation-intensive likelihood. The discussion of model fits and sensitivity of Hack et al. summarizes important features of the posterior inference.

Variability

An important element of variability for the reported risk assessment is the choice of dose metric. The PBPK model provides a comprehensive probabilistic description of all metabolites in all specified compartments. The Markov chain Monte Carlo implementation allows easy inference about any event of interest. In particular, for any tentative dose metric the model includes inference about variation with dose and correlation with other tentative dose metrics. Although the PBPK model cannot deliver a decision on the choice of dose metric, it can simplify the decision by describing the joint distribution of possible dose metrics. The committee recommends that the investigators consider a moderately large set of possible dose metrics, including the metrics described earlier in this chapter, and report the correlation of those metrics over different exposure and inhalation concentrations. Hack et al. (in press) include results on correlation of dose metrics and parameters and suggest that parameters that have little impact on the predicted dose metrics are less critical for risk assessment.

FINDINGS AND RECOMMENDATIONS

EPA's use of the Fisher (2000) and Clewell et al. (2000) PBPK models for trichloroethylene in its 2001 draft risk assessment was reasonable given the available data for liver and kidney cancer. The committee supports the inclusion of multiple dose metrics including AUC, C_{\max} , and lifetime average daily dose, as it is not clear which is the most appropriate dose metric for a given end point.

EPA relies on the description by Bois (2000a,b) of uncertainty in the Fisher (2000) and Clewell et al. (2000) models. This includes updating uncertainties by using the paradigm of Bayesian inference and implementation by Markov chain Monte Carlo posterior simulation. Bois' extension to population models captures an important aspect of the variability. A joint probability model for all relevant quantities (concentrations in different tissue compartments) implies a coherent description of the variability across different dose metrics.

None of the currently available PBPK models considers all possible routes of trichloroethylene exposure (e.g., dermal) or dose metrics for all adverse health effects (e.g., neurotoxicity). The harmonized model is a reasonable extension of the Fisher and Clewell models and is a step in the right direction, but the mode of action and appropriate dose metric are not clear for each end point. PBPK models do not resolve the uncertainty about the mode of action, but they can inform experimental designs for studying the mode of action. Moreover, understanding the mode of action drives PBPK model elaboration.

Recommendations:

- Because there is potential for trichloroethylene exposure via dermal absorption, the committee recommends that future PBPK models used for trichloroethylene risk assessment include a description of dermal absorption similar to the approach of Poet et al. (2000).
- The committee recommends additional studies to evaluate how well alternative dose metrics predict toxic response. The model could be used to investigate alternative study designs. For example, one could simulate liver concentrations of trichloroacetic acid in several different groups of laboratory animals that receive the same lifetime average daily dose by different dosing regimens to compare the lifetime average daily dose with an internal dose metric (that is, trichloroacetic acid concentration or AUC in liver). One group of subjects could receive intermittent high exposures to trichloroethylene and another group could receive lower daily doses; some groups may receive the same daily dose by different routes (e.g., inhalation versus drinking water). Carrying out the corresponding studies in laboratory animals would facilitate the desired comparison of alternative metrics with respect to their ability to predict the toxic end point.

The PBPK models used in the 2001 draft risk assessment focused on liver (Fisher) and kidney (Clewell) cancer. End points not addressed by currently available PBPK models include reproductive and developmental toxicity, neurotoxicity, immunotoxicity, and others.

Recommendation: PBPK models should be developed for other toxicity end points, such as neurotoxicity and developmental outcomes.

- Simmons et al. (2002) and Keys et al. (2003) included a brain compartment in their models for trichloroethylene, which could be used to predict target organ doses relevant to neurotoxicity in future generations of PBPK models used for trichloroethylene risk assessment. The committee recognizes that there may be little or no data available to confirm model predictions for brain tissue concentrations of trichloroethylene and metabolites in humans. However, including all relevant uncertainties is key and can be formalized under Bayesian inference and implemented with the Markov chain Monte Carlo approach used by Bois (2000a, b). Description of uncertainties in prior simulation might indicate that the approach is not practical without collecting additional data.

- Fisher and others have incorporated developmental exposure in utero and via lactation in their PBPK models for perchlorate (Clewell et al. 2003a,b; Fisher et al. 2000); this approach could be applied to trichloroethylene to investigate dose metrics relevant to developmental effects of trichloroethylene exposure. See Chapter 9 for additional guidance on producing developmental PBPK models.

None of the PBPK models for trichloroethylene describes the effect of exposure to chemical mixtures that include trichloroethylene. For example, ethanol and trichloroethylene share enzymatic pathways of metabolism.

Recommendation: A combined PBPK model for trichloroethylene and ethanol would enable investigation of exposure to this mixture. This approach could be used for other mixtures with shared metabolic pathways or common metabolites. A similar approach could be taken to include the effect of disease states on trichloroethylene disposition (e.g., induction of CYP2E1 in diabetes).

In summary, pharmacokinetic models can be useful tools to identify data gaps and research needs to reduce uncertainty in risk assessment. More data and a better understanding of the mode of action for various end points are needed for a revised trichloroethylene pharmacokinetic model, in conjunction with appropriate pharmacodynamic models, to be useful for further understanding the risks posed by trichloroethylene.

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Issues in the Assessment of Dose Response

The assessment of dose-response relationships is used to predict the incidence, probability, or magnitude of an adverse health effect in an individual or population for any amount of exposure to a toxicant. Dose-response relationships can also be used to estimate an exposure concentration or range of concentrations likely to correspond to a specific probability or risk of adverse health effects (e.g., dose corresponding to 10^{-6} excess risk of cancer). These assessments should include quantitative descriptions of the uncertainty of those predictions, such as statistical confidence limits or sensitivity analyses in which assumptions used in the analysis are varied. Sensitivity to assumptions is of particular concern with epidemiologic data because of the potential effects of measurement error and uncontrolled confounding (Lash and Fink 2003). The U.S. Environmental Protection Agency (EPA) draft health risk assessment on trichloroethylene used various approaches to assess dose-response relationships for cancer and non-cancer health effects, including point-of-departure methods, linear extrapolation, and nonlinear modeling (EPA 2001b). This chapter discusses those approaches and their application to trichloroethylene.

POINT OF DEPARTURE DETERMINATION

Non-cancer Effects

The point of departure is a dose estimate developed from experimental or observational data on cancer or non-cancer effects. For non-cancer dose-response data, the point of departure has generally been defined as the no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or a modeled dose corresponding to an incremental effect (e.g., the lower 95% limit of the dose or concentration corresponding to a 10% increase in response [LED₁₀ or LEC₁₀]). Use of NOAEL and LOAEL has been criticized because of their dependence on features of the experimental design of the study from which they are derived (e.g., spacing of the dose groups) and their lack of consideration of statistical error or the shape of the dose-response curve (Crump 1984). The NOAEL and LOAEL provide only a single summary statistic and are of limited use in describing the quantitative dose-response relationship.

Continuous dose-response models are thus preferred (Faustman and Bartell 1997). However, not all dose-response data sets are suitable for estimating parameters in continuous dose-response models. At least three dose groups are required for continuous dose-response modeling, whereas studies with as few as one or two dose groups can sometimes be used to identify a NOAEL or LOAEL. In its draft risk assessment for trichloroethylene, EPA (2001b) compared a variety of NOAEL, LOAEL, and LED or LEC values calculated from different dose-response data sets and converted to human-equivalent doses by various approaches. Points of departure for inhalation dosing and for oral dosing were then selected from among the lower NOAEL, LOAEL, and LED₁₀ or LEC₁₀ values for each route of exposure.

The committee found that determining points of departure for non-cancer end points in EPA's draft risk assessment for trichloroethylene was generally consistent with common practice and the dose-response evidence available at the time of the assessment. However, several points should be addressed in the future. First, the criteria used to determine when toxicologic or epidemiologic data are suitable for continuous dose-response modeling should be specified. Second, the rationale for choosing a 10% response level should be provided, and presenting results for several other response levels should be considered. The ability to quantify specific response levels depends on the study design, which often differs in epidemiologic and toxicologic studies. Third, the dose-response model(s) used to estimate LEDs should also be presented. Fourth, the methods used to derive human-equivalent doses from animal data should be described. It is important that the summary statistic used for the conversion (e.g., area under the curve or peak values) be provided and be readily apparent (not placed in footnotes or separate documents). Given the variety of approaches available to derive human-equivalent doses, the results using the different approaches should be presented in tables that allow them to be easily identified and compared. This suggests that multiple dose metrics should be considered for each data set to help inform the selection of the appropriate adjustment methods.

Cancer Effects

For cancer dose-response data, the point of departure is an estimated dose "near the lower end of the observed range without significant extrapolation to lower doses" (EPA 2005a, p. 1-13). Guidance for performing such dose-response assessments is provided in EPA's new cancer guidelines. These guidelines were finalized after the agency conducted the risk assessment on trichloroethylene, so EPA will need to update the assessment of trichloroethylene to ensure that it is consistent with the new guidelines. For example, with the exception of its consideration of kidney cancer, EPA (2001b) proposed the use of only LED₁₀ values from rodent carcinogenicity studies (adjusted to achieve human-equivalent doses) as points of departure in the trichloroethylene assessment. The new cancer guidelines now suggest that estimated doses corresponding to a 1%, 5%, and 10% increase in response (LED₀₁, LED₀₅, and LED₁₀) should be presented routinely and considered as potential points of departure and that central estimates as well as lower confidence bounds for estimated doses be presented. It has been reported that the LED₀₅ estimate is close to the NOAEL for many conventional bioassays with continuous response variables and that the NOAEL exceeds the LED₁₀ estimate for many bioassays with quantal response variables (Allen et al. 1994).

It is important to explain and justify the procedure for selecting the particular response level for the point of departure so that the selection does not seem arbitrary. One procedure for

choosing from among the 1%, 5%, and 10% response levels could be to select the highest response level exceeded by the lowest observed response level among all exposed dose groups. For example, consider a study with doses of 0, 100, 200, and 300 mg/kg/day and observed excess risk of 0%, 8%, 14%, and 20%, respectively. Using the suggested criterion, 5% excess risk would be selected as the response level for the point of departure, as it is the highest among the two options below 8%. A different approach may be necessary when most exposed individuals have unique doses (common in epidemiologic studies). Categorization of exposure in quartiles or other groupings may be helpful in that situation, but the results may be sensitive to the arbitrary cut points used to distinguish categories, just as NOAELs are sensitive to cut points (Bailer et al. 1997), so an explicit procedure should be specified. This procedure should be objective and transparent and should yield a point of departure near the lower end of the range of tested non-zero doses in accordance with EPA guidelines. Other procedures may also be reasonable, so EPA should establish a clear protocol.

Under the current cancer guidelines, a variety of dose-response models may be used to estimate effective doses (EDs) and LEDs from the data. Although the logit and probit models typically used for these estimates should provide similar ED values for any given level of response, their LED values may be more divergent. If the establishment of point-of-departure-based dose-response assessment as a default policy model is intended to avoid the difficulties of choosing from among equally reasonable scientific models, it would be sensible to stipulate a default modeling procedure rather than allowing for a variety of approaches.

The effects of selecting different dose metrics for adjustment to equivalent human doses from animal models may be important for both non-cancer and cancer dose-response modeling. For example, EPA notes that subchronic dosing studies indicate that cumulative exposure metrics may not be appropriate for predicting the risk of liver cancer (EPA 2001b, p. 4-20), but it did not evaluate the fit of cumulative exposure metrics for other end points.

LINEAR EXTRAPOLATION FROM THE POINT OF DEPARTURE TO ZERO DOSE

EPA's cancer guidelines state that "linear extrapolation should be used when there are [mode of action] data to indicate that the dose-response curve is expected to have a linear component below the [point of departure]. Agents that are generally considered to be linear in this range include agents that are DNA-reactive and have direct mutagenic activity, or agents for which human exposures or body burdens are high and near doses associated with key precursor events in the carcinogenic process" (EPA 2005a, p. 3-21). When the mode of action is unclear, EPA suggests that linear extrapolation (or interpolation¹) be used as a default approach, as it is thought to overestimate the response level for a given dose. EPA guidelines support the presentation of results from more than one approach when alternative models have "significant biological support" or when multiple modes of action appear to exist. In the draft risk assessment on trichloroethylene, the low dose-response function was estimated by extrapolating

¹The committee used "extrapolation" to describe the process of modeling between the point of departure and zero, because conventionally this term has been applied to the process. However, "interpolation" is a more accurate description when the modeling process is applied to data with a zero dose group. Most toxicologic and epidemiologic data sets include a control group or some observations at zero dose, in which case modeling between the point of departure to zero dose is an interpolation between two points. This is true even after adjusting for background response using excess risk or other risk metrics, although the data point at the origin is sometimes excluded from the dose-response plot (e.g., NRC 1983).

between zero dose and the point of departure. The committee found this approach to be consistent with the current cancer guidelines. Because the mode of action for carcinogenicity is unclear and may include multiple pathways (see Chapters 3 and 4), EPA's presentation of results from both linear and nonlinear model approaches is also appropriate.

The committee recommends that a general study of the implications of linear extrapolation from the point of departure for dose-response assessment be performed in support of all human health risk assessments (not just for trichloroethylene). Such study is warranted because the statistical properties of linear extrapolation between zero dose and the point of departure have never been evaluated, unlike the statistical properties of traditional dose-response modeling techniques such as probit and logit regression (McCullagh and Nelder 1989). Such evaluations typically include mathematical derivations or simulation studies to determine the degree of conservatism compared with hypothetically true dose-response models as well as comparisons among alternative dose-response models using real data sets. If the true shape of the dose-response curve is sigmoidal, the linear extrapolation will likely overestimate the actual risk at a given dose, as suggested by EPA (2005a; p. 3-21), but the validity of that claim and the extent of overestimation are difficult to evaluate without explicitly defining the point-of-departure selection procedure.

Although the linear extrapolation procedure was adopted to avoid the difficulty of choosing from among alternative dose-response models that fit equally well, there appears to be little scientific basis for evaluating its performance. The claim that "the dose-response curve for [trichloroacetic acid] appears linear" (EPA 2001b, p. 4-20) is weak, as it is based on only three data points, two of which appear to fall above the point of departure. The relevant issue is whether the dose-response curve is linear below the point of departure, but there appear to be insufficient data to evaluate this claim for human exposure to trichloroethylene or trichloroacetic acid.

Although linear extrapolation has been advocated as an intentionally conservative approach to protect public health, there are some theoretical reasons to think that sublinear nonthreshold dose-response models may be more relevant for human exposure to toxicants, regardless of the mode of action. One basis for judging that dose-response patterns are not linear is related to how population variability affects the dose-response curve for humans. For example, a possible interpretation of mechanistic data on trichloroethylene for renal cancer is that any individual may have an exposure threshold below which the glutathione conjugation pathway may be less utilized; at an exposure below that threshold, there is possibly no excess risk of an individual developing renal cancer. However, the existence of individual dose-response thresholds does not necessarily imply the existence of a population dose-response threshold below which nobody is at excess risk of renal cancer; in fact, most plausible models for variability in individual dose-response thresholds imply a sigmoidal population dose-response curve even in this case. The flattening and smoothing effects of population variability on the shape of the population's dose-response curve have long been recognized for the deterministic model in which each individual has a tolerance to an exposure and the tolerance values have a Gaussian, logit, or other typical distribution (Dobson 1990), but similar results hold for many alternative models. The discussion above does not account for measurement error, which can "linearize" nonlinear dose-response relationships.

To understand this, consider a general function, $\pi_i(d)$, describing the probability of a specific toxic response in an individual, i , given a dose, d . The probability of response in an

individual randomly selected from a population of n individuals is then given by $\sum \pi_i(d)/n$. The classic tolerance model may then be expressed as:

$$\begin{aligned}\pi_i(d) &= 0, \text{ if } d < \theta_i \\ \pi_i(d) &= 1, \text{ if } d > \theta_i\end{aligned}$$

where θ_i is the tolerance for individual i . Although this model describes a dose-response threshold for any individual, the shape of the dose-response curve for a population of individuals is described by the cumulative distribution of values for θ_i . For example, it is well known that a Gaussian distribution for θ produces a probit dose-response model for a population, and a logit distribution for θ produces a logistic population dose-response model (Dobson 1990). One might expect these individual tolerances to vary extensively in humans depending on genetics, coincident exposures, nutritional status, and various other susceptibility factors, producing a continuous distribution with one or more modes and relatively narrow tails describing the population extremes. In contrast, a uniform distribution of tolerance values is required to produce a linear dose response under this model. Under realistic assumptions, the dose-response curve is sublinear below the 10% response level, with only approximate linearity at extremely low doses.

Consider a more complicated model that allows for increasing risk with exposure above the individual threshold in an approximately linear fashion, as one might posit for a mode of action that takes effect only at higher doses:

$$\begin{aligned}\pi_i(d) &= 0, \text{ if } d < \theta_i \\ \pi_i(d) &= 1 - \exp[-\beta(d - \theta_i)] \text{ if } d > \theta_i\end{aligned}$$

where β represents the effect of exposure about an individual's threshold. Although this function produces a classic "hockey-stick" dose-response shape for any individual (Figure 12-1), the same model produces a sigmoidal population dose-response curve with no threshold, assuming among the exposed population a Gaussian distribution for θ (Figure 12-2). Although these dose-response models are just two simple examples, a similar phenomenon of translating individual dose-response functions to population dose-response functions should be considered for any human dose-response assessment. It is important to emphasize that it is the population-based dose-response relationships that are generally observable, not individual dose-response relationships, and population dose-response functions form the basis for public health interventions and regulations. The population dose-response model may take various forms depending on the mode of action and distribution of susceptibility factors among individuals, but both linear dose-response relationships and population thresholds are difficult to derive without resorting to uniform distributions of individual susceptibility factors such as θ .

There is epidemiologic evidence for some toxicants (other than trichloroethylene) suggesting a linear or even a supralinear dose response at low doses in humans (Stayner et al. 2003). Although these data may simply reflect unusual mechanisms or heavy-tailed population distributions of susceptibility, the effects of exposure measurement error can distort the apparent shape of an observed dose-response curve. This is an important difference between epidemiologic and toxicologic studies; the latter tend to have relatively little exposure measurement error because of intentionally administered doses are used. An additional issue that can affect the shape of an agent's dose response curve is background effect from spontaneous

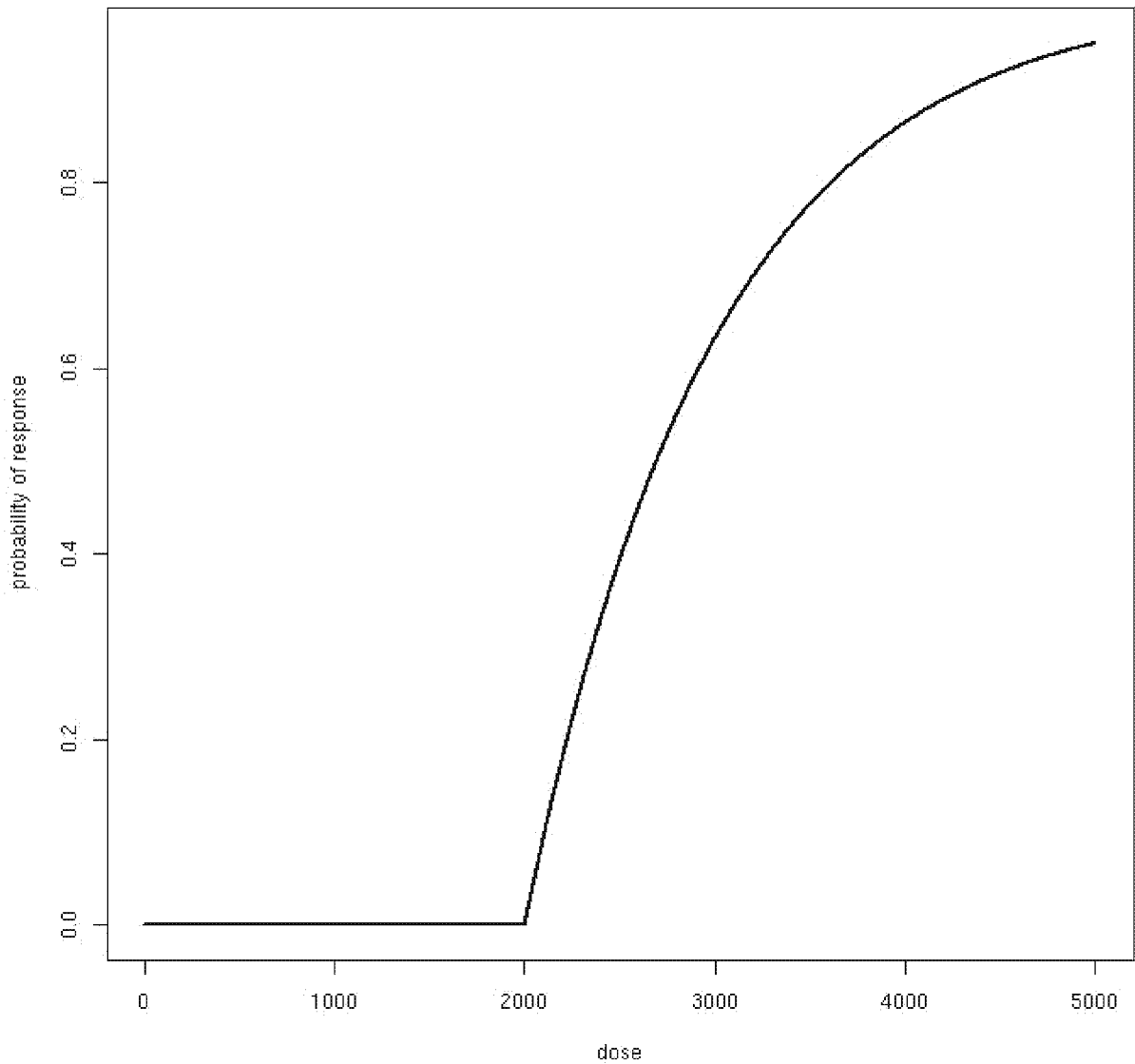


FIGURE 12-1 Classic “hockey-stick” dose-response shape.

occurrence or exposure to other chemicals acting by the same mode of action. If background effects can be assumed to be additive in a mechanistic manner, it would shift the dose-response curve so that response to any additional exposure is linear (Peto 1978; Hoel 1980; Crump et al. 1976; Lutz 1990; Clewell and Crump 2005).

This discussion illustrates an important fact: population variability is an inherent feature of the dose-response curve. Moreover, variability in one parameter could affect the shape of the dose-response curve differently than variability in another parameter (e.g., θ versus β), depending on the underlying probability function $\pi_i(d)$ as well as the shape and location of each population distribution. Therefore, it is difficult to draw conclusions about the shape of the dose-response model from the mode of action alone, without any information on response variability among humans. In fact, any monotonic dose-response model, including the linearized multistage

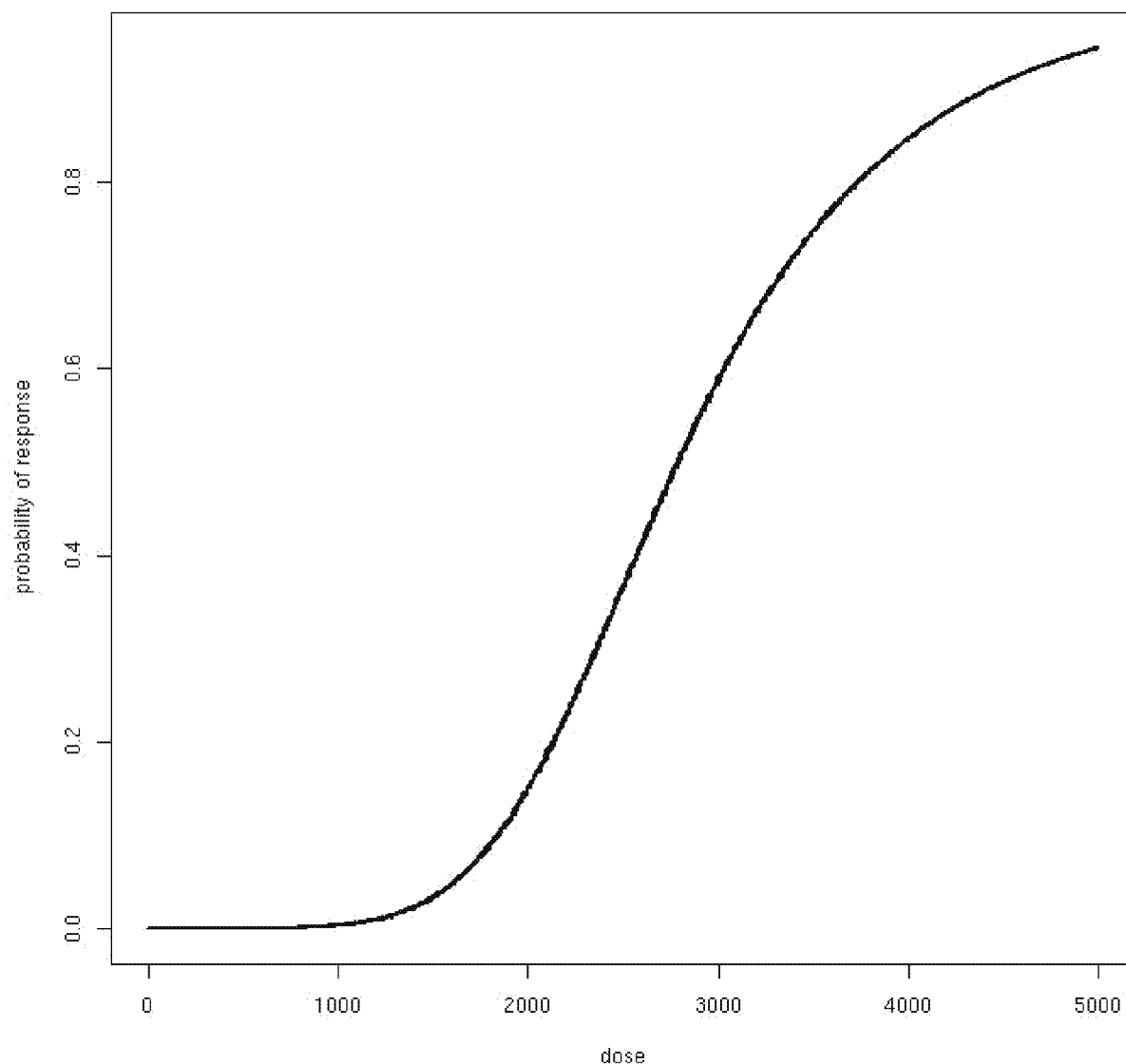


FIGURE 12-2 Sigmoidal population dose-response curve with no threshold.

model, can be defined solely in terms of a tolerance distribution without resorting to mechanistic arguments. These considerations suggest that one must consider both the role of mode of action and the role of response variability among humans in determining the likely shape of the dose-response function.

From a scientific perspective, one approach to characterizing dose-response relationships is to develop models that explain the variability in the available data and, when possible, incorporate known mechanisms of toxicity. Population variability can be directly incorporated within these models by using hierarchical model structures (Allen et al. 1994; Leroux et al. 1996; Gelman et al. 2004; Longnecker et al. 2005) instead of arbitrary uncertainty factors. Although direct measurements of population variation in human susceptibility are rarely available, the relevant parameter(s) could be statistically estimated along with any other parameters in the dose-response model. Alternatively, a surrogate such as variation of rates in a key

toxicodynamic step could be used to estimate population variation in susceptibility. Formal Bayesian methods similar to those applied for physiologically based pharmacokinetic modeling of trichloroethylene offer a natural unified framework for addressing population variability and uncertainty in dose-response assessment and for incorporating information from multiple sources (see Chapter 11). Explicit modeling approaches eventually might replace post hoc applications of uncertainty factors for both cancer and non-cancer dose-response assessment.

From a public health perspective, the optimal dose-response model for any toxicant is often unclear, requiring the judicious use of plausible models that adequately protect health. Moreover, typical toxicologic and epidemiologic data rarely provide confirmation for potentially susceptible subpopulations, such as children, the infirm, and other subgroups, suggesting that, in the face of uncertainty, appropriate correction factors should be applied to protect the population from unnecessary risks.

ALTERNATIVE DOSE-RESPONSE FUNCTIONS

A number of alternatives to point-of-departure-based approaches are also presented for cancer end points in EPA's draft health risk assessment for trichloroethylene, including mechanistic models and linear models for several epidemiologic data sets. The linear models are cursory and in some cases could be improved with more realistic dose-response models, given the original study data. However, exposure ascertainment is weak in many of the epidemiologic studies, as discussed in Chapter 2 and in the EPA assessment, so it may not be worthwhile to conduct more detailed dose-response modeling for many of these data sets.

The committee endorses the general use of epidemiologic data in dose-response assessment but notes that the exposure assessments in most studies may not be of sufficient quality to use for these purposes. Committee members agree that epidemiologic data for trichloroethylene should be evaluated and described more fully than was done in the EPA (2001b) draft risk assessment, giving more weight to data sets of higher quality in the overall evaluation (see Chapter 2). The relative merits and modeling assumptions used in each epidemiologic dose-response assessment should also be clearly delineated; in some cases, it was difficult for committee members to understand how particular epidemiologic data sets were used for dose-response modeling of trichloroethylene.

The committee also endorses EPA's exploration of hypothetical mechanism-based models such as the two-stage cancer model. However, for current pharmacokinetic models for trichloroethylene, the two-stage model is not well validated and should be viewed only as a plausible alternative to other nonlinear dose-response models. Parameterization of mechanistic models is often difficult, and it is important to fully describe the details of the model.

OTHER ISSUES

Definitions of empirical dose-response models, benchmark dose models, and mechanism-based dose-response models given in the EPA draft risk assessment (p. 4-01) are oversimplified. The report groups empirical dose-response models with benchmark dose models and draws too fine a distinction between those models and mechanism-based dose-response models. Although this may seem to be a minor point, it suggests that little or no consideration was given to

approaches that combine mechanism-based model structures with empirical estimation. Any dose-response model can be used to estimate a benchmark dose, even if it is mechanism based. Moreover, mechanism-based models can be parameterized with experimental measurements of individual parameters, “curve-fitting” (that is, statistical estimation using empirical dose-response data), Bayesian analysis synthesizing experimental measurements and dose-response data, or by a combination of these approaches (Leroux et al. 1996; Sherman and Portier 1997; Dunson et al. 2004). Finally, even the parameters for simple logit and probit dose-response models used for curve fitting have biological interpretations, albeit limited ones. It may be best to view dose-response model structures as a continuum from less detailed to more detailed biological information and estimation of parameters in models of dose-response relationships as a separate issue that can be tackled through direct measurement of individual parameters, statistical curve fitting, or Bayesian combination of the two approaches.

UNCERTAINTY ANALYSIS

Uncertainty analysis is the process of providing a description of uncertainty surrounding quantitative estimates of risk. The simplest form of uncertainty analysis is to provide a qualitative description of the sources of uncertainty and their potential effects on the risk estimates. Quantitative assessments of uncertainty can be provided by techniques such as interval analysis and probabilistic analysis. These techniques attempt to predict a range and likelihood of plausible risk estimates rather than a single estimate of the magnitude of risk. In January 2006, the Office of Management and Budget (OMB) released a proposed risk assessment bulletin that states that

When a quantitative characterization of risk is made available, this should include a range of plausible risk estimates, including central estimates. A “central estimate” of risk is the mean or average of the distribution; or a number which contains multiple estimates of risk based on different assumptions, weighted by their relative plausibility; or any estimate judged to be most representative of the distribution. The central estimate should neither understate nor overstate the risk, but rather, should provide the risk manager and the public with the expected risk.

Although formal quantitative uncertainty analysis techniques are commonly applied in the exposure assessment and pharmacokinetic modeling portions of environmental risk assessment, they are not yet widely used for dose-response modeling (Presidential/Congressional Commission on Risk Assessment and Risk Management 1997). Such applications are substantially different than traditional regulatory approaches such as the application of safety/uncertainty factors and intentionally conservative assumptions such as upper bound dose-response estimates. The proposed OMB bulletin suggests that formal quantitative approaches will be applied routinely in future EPA’s risk assessments, including revisions to EPA’s trichloroethylene assessment. Below the committee uses the review by Bartell (2005) to summarize some of the quantitative techniques for performing uncertainty analyses.

Interval Analysis

Interval analysis involves estimating the risk twice, using best-case and worst-case scenarios to identify a range (Alefeld and Herzberger 1983; Ferson 1996). While this is a straightforward and easily understood approach, it does not provide information about the relative plausibility of individual risk estimates within the interval. For example, it does not indicate whether each point in the interval is equally likely or whether estimates near the center of the interval are more likely than the estimates near the ends of the interval. Furthermore, single points for base-case and worst-case scenarios may be difficult to define.

Statistical confidence intervals and prediction intervals are another type of interval analysis. These intervals are based on frequentist or Bayesian statistical methods, and attempt to capture the true risk estimate with a fixed confidence level (e.g., 95%) (DeGroot 1989). When traditional frequentist methods are applied, model parameters are usually divided into what is known and unknown, and parameters that are partially understood or for which educated guess may be made are not considered. As an alternative, Bayesian methods offer the advantage of being able to handle such complexities (Greenland 2001).

Probabilistic Analysis

Probabilistic analyses are used to describe risk using one or more probability distributions to indicate the plausibility of an entire range of risk estimates. The most common method is to use Monte Carlo simulation after the initial quantitative risk assessment. The approach involves selecting probability distributions to represent uncertainty in the model parameters. Parameters that are dependent on one another may be specified by such techniques as multivariate distributions, conditional distributions, and rank correlations. Using the specified probability distributions and correlation structure, plausible sets of parameter values are randomly and repeatedly selected. The risk estimates calculated for each set of parameters (tens of thousands or hundreds of thousands) approximate the distribution of uncertainty regarding the risk. Thus, the Monte Carlo distribution is thought to present the range and relative plausibility of various risk estimates. However, cautions have been raised about whether the relative plausibility of an entire range of risk estimates can ever be determined reliably and the possibility of misleading risk managers (Ferson 1996). Errors in uncertainty propagation can be introduced when correlations between parameters are inadequately characterized or are overlooked.

FINDINGS AND RECOMMENDATIONS

The key scientific issues related to the dose-response assessment for trichloroethylene include selection of the data to be used, selection of the point of departure for low-dose extrapolation, methods for modeling from the point of departure to zero dose, and characterization of uncertainty and variability in estimates of cancer and non-cancer risk.

Although it is preferable to use continuous dose-response models to identify a point of departure for non-cancer risks, the committee recognizes that suitable data on trichloroethylene were not always available for such modeling. Therefore, a NOAEL or LOAEL may be used when a continuous dose-response model cannot be developed to determine LEDs. The selection

of NOAELs and LOAELs is relatively straightforward, but modeled estimates require more explanation and justification.

For dose-response assessment for risks of cancer, EPA's guidelines call for selecting a point of departure from among modeled doses near the lower end of the observed range. A number of response levels and dose metrics are available for performing such assessments, and it is important that all relevant ones are considered and that a clear rationale is provided for selecting the point of departure.

Recommendations:

- Several points of departure should be considered and compared when performing point-of-departure-based dose-response assessments for cancer and non-cancer end points.
- When modeled estimates are used as points of departure in cancer and non-cancer risk assessments, it is important that (1) criteria are established for determining what data sets are suitable for modeling, (2) the selected response level (e.g., 10%) is justified or multiple response levels are modeled and compared, (3) dose-response models are clearly described, (4) different dose metrics are considered and compared to assess whether the choice of metric substantially affects the dose-response assessment, and (5) the methods for estimating human-equivalent doses are specified when animal data are modeled.

There are several approaches to extrapolating from the point of departure to zero, including linear and nonlinear methods. Much emphasis has been given to incorporating mode-of-action information on the carcinogenicity of trichloroethylene into such extrapolations. The committee recommends that information on both the mode of action and on response variability among humans be used to clarify the shape of the low dose-response curve. The mode of action for trichloroethylene as a kidney carcinogen remains unclear and likely involves multiple pathways.

Recommendations: There appear to be insufficient epidemiologic data to support quantitative dose-response modeling for trichloroethylene and cancer. The committee recommends that toxicologic data be used to fit the primary dose-response model(s) and that the available epidemiologic data be used only for validation. The committee does not believe that the available information is sufficient to determine the best dose-response model for trichloroethylene. The default linear extrapolation procedure suggested in the EPA cancer risk assessment guidance can be applied but should first be explicitly defined.

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